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TITLE: UG311, an Oncofetal Marker Lost With Prostate Cancer Progression

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Purpose: The mRNA expression of a paralogous human sequence to UG311, a murine urogenital sinus expressed sequence tag, was found to decrease with androgen independent progression in the LNCaP model of human prostate cancer progression as determined by RNA blotting. Analysis of the sequence of UG311 determined significant homology to a single-stranded nucleic acid binding protein, nmt55. To exclude nmt55 as the gene corresponding to UG311, antibodies and cDNAs were acquired.
Scope: As members of the single-stranded nucleic acid binding protein family, nmt55 and UG311 may play a role in DNA repair or RNA splicing. Both functions are currently under evaluation for nmt55. Either of these functions is likely to have impact on the progression and potential therapeutic outcome for prostate cancer patients. Improper splicing would lead to entire classes of proteins being disrupted. Loss of DNA repair enzymes would lead to increased genomic instability and accelerated progression.
Major Findings/progress: We have attempted to focus on the cloning of the UG311 paralog w/ a minor emphasis on completing the exclusion of nmt55 as the UG311 paralog. The UG311 insert was used as a high stringency probe to screen the C4-2 lambda ZAP library. Ten strong plaques after two rounds of purification were analyzed for the size and sequence of the insert acquired. All inserts matched to the NONO sequence. This is a candidate as it shows significant homology to UG311 insert sequence. Unfortunately, this sequence shows an increase expression in the human prostate cancer cell lines as compared to previous data from UG311. Several additional UG sinus clones were used to screen the C4-2 library to clone additional cDNAs from other UG sinus genes that have altered expression in the human prostate cancer cell lines model of progression. Since nmt55 is a nuclear protein it was felt inappropriate to screen by whole cell lysate, therefore, nmt55 antibodies were used to screen nuclear extracts of prostate cancer cells. The results confirm the RNA expression profile of the nmt55 gene.

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Introduction/overview

During this reporting period my laboratory has endured rather rapid personnel turnover. Margaret C Shea, B.A. was the research technician on this project at the start of the reporting period. In May she resigned to take a job more suited to her environmental consciousness. She was replaced by Barbara Szomju, B.A. Ms. Szomju has developed admirably in the last nine months, however, significant down time was incurred as the result of replacing Ms. Shea and training Ms. Szomju. Further, I had managed to acquire a Graduate Research Associate during the first part of last year, Dr. Brian E. Nicholson. He chose to relocate to another lab on the advice of my Chairman who knew I would be relocating to another University. Finally, I have been in the middle of a Job Search that has resulted in the loss of significant effort and mental concentration on the project at hand. I anticipate that my relocation to the University of Delaware this June will help to alleviate many of these problems.

The subject of the current research is to discern whether UG311 and nmt55 are the same gene. Preliminary sequence analysis indicated a large degree of homology over the area of the UG311 expressed sequence tag (EST), however, the RNA size for UG311 is different than that reported for nmt55 as described in the original application. Since reagents are available for nmt55 then the pattern of expression will be determined to see if it is the same as UG311. If not, then UG311 is likely to be a novel member of this family and warrants extensive investigation. The purpose is to determine the expression pattern and the association of nmt55 and UG311 in prostate carcinogenesis and progression. Since an mRNA corresponding to UG311 has reduced expression in aggressive prostate cancer cell lines, this gene will be re-expressed in aggressive prostate cancer cells to study its ability to normalize the cancer cell phenotype. The ultimate scope of this work is to develop new prostate cancer biomarkers or therapeutic targets.

Body

Aim 1e. RACE reactions. These data were included in last year's report but the sequences were analyzed subsequent to this submission and form the basis of their inclusion here. As shown in figure 1, 5'- and 3' RACE reactions have been optimized for control reactions. Furthermore, candidate bands have been gel purified, cloned into pCR2.1-TOPO (a PCR product cloning vector). Several of the bands have been pulled, cloned, amplified and sequenced. Sequence of RACE bands can be found in figure 2a. Top matches to GenBank alignment are shown in figure 2b. The GenBank comparisons and alignment are part of aim 1f. As shown in figure 2b none of the BLAST alignments for the RACE bands correspond to any of the potential UG311 orthologs, namely nmt55, p54nrb or NonO-Non-pou octamer (NONO) binding protein on the X chromosome. Further, none of these alignments are particularly useful or informative. This is possibly due to the quality of the input sequence obtained from the RACE clones and resulting poor sequence quality output for BLAST search.

Recent BLAST searches of the human genome using UG311 as a query resulted in matches to both chromosome 16 and chromosome X. nmt55, p54nrb and NONO are closely related splice variants. There also exist a few other splice variants of the NONO-like sequences at this locus. Additionally, the blast hit fell in the middle of a predicted ORF that is different than the NONO-like sequences. The chromosome 16 locus contains 2 potential ORFs that encompass the UG311 BLAST homology region which are also quite similar to the NONO locus on the X-chromosome. However, this locus is only putative since no mRNA or cds have actually matched to this area. In short , there are still several possibilities in these loci that could be responsible/explain the loss of expression of a UG311-related mRNA with prostate cancer progression as observed on the LNCaP series of cell lines.

Aim 2a. Immunocytochemistry/immunohistochemistry of human cell lines and tissues. The object of this aim is to determine if nmt55 has the same expression pattern as UG311, namely decreased expression with prostate cancer progression. These data were presented in the first annual progress report. Staining of human prostate cancer cell lines showed no change in intensity with the nmt55 antibodies and good evidence of nuclear localization of the protein. These data suggested that UG311 was a different gene. Protein expression data were not available as of the last report. Given the possible discordant expression of RNA and protein and potential artifact associated with IHC, it was deemed necessary to do western blotting. Further, since the NONO-like proteins are nuclear localized we had to compare nuclear extracts. We have managed to get western blots of nmt55 from KCl-extracted nuclei of prostate cancer cell lines. This technical skill was not available to my laboratory in year one.

Nuclear extracts and western blots for nmt55 were isolated by KCl extraction of nuclei as described in Current Protocols [1]. Thirty micrograms of nuclear extract were used per lane for each of the cell lines, LNCaP, LNCaP-SF (serum-free growth), C4-2 and PC-3. These extracts separated under denaturing SDS-PAGE using the NOVEX gel system and bis-bicine buffer system (NuPage conditions). Samples were transferred onto nylon reinforced nitrocellulose filter paper in TOBIN buffer plus 20% methanol. The blots were then blocked in PBS containing 5% non-fat dry milk and 0.1% Tween -20 overnight at 4 degrees celsius. NMT antibodies were then used as follows, nmt-4 at 1: 1500, nmt-5 at 1:2000 and nmt-1 at 1 :2500 as recommended by Robert Moreland, Ph.D. Primary antibodies were incubated for 1 hour at RT followed by incubation with either anti-rabbit-HRP or anti-mouse-HRP at 1:5000 dilution for 1 hour at RT. ECL (Amersham Biosciences) was used to detect immunoreactive bands. The results are shown in figure 3. The nuclear extracts demonstrate that nmt55 (NONO-like) actually increase with serum withdrawal in LNCaP and with progression in the LNCaP/C4-2 pair of cells. The unrelated PC-3 cells, however, express less nmt55 than either LNCaP or C4-2 and are more tumorigenic than LNCaP but less metastatic than C4-2 cells. Therefore, it is still possible that nmt55 (or other NONO-like sequences) may play a role in the metastatic process but the expression pattern is not the same as that observed previously for UG311, namely a decrease in expression in the LNCaP progression model.

Aim 1a. In collaboration with Fan Yeung (graduate student in Urology/Biochemistry) a Lambda-ZAP library has been constructed from C4-2 cells. The advantages of using Lambda-ZAP (Stratagene Inc, La Jolla, CA) are 1) Larger insert size (12kb max) 2) both prokaryotic and eukaryotic expression and 3) rescue to plasmid. C4-2 was chosen to decrease the number of positive colonies upon hybridization with UG311-est sequences. The library has been titered at >1.1 x 10^7 pfu/µl phage. The following UG sinus EST inserts were chosen for screening, UG311, UG101, UG102, and UG307 based on the changes in the expression patterns between LNCaP and its androgen-independent derivative. Several UG-ESTs were chosen because it is just as simple to screen a phage library with one insert as it is to do 4 inserts. Screening of Phage libraries to clone a full-length UG311 paralog is described in Aim 1b. Aims 1c-d require the results of aim 1b, therefore, the generation of clones expressing sense and antisense UG311 has not been accomplished to date due to the lack of confidence in having the human UG311 paralog. **Note Bene**: the use of Lamda-ZAP system also satisfies portions of aim 1g where the insert was to be cloned into separate prokaryotic and eukaryotic expression vectors.

Figure 4 shows the plasmids rescued after two rounds of screening with UG311 as a probe. Approximately 300,000 clones were screened in round 1. Four plaques were pulled from round one for amplification and rescreening. Eight, well separated plaques were pulled for rescue. These minipreps, figure 4a, indicate at least 2 and maybe 3 sizes of insert. Restriction digests confirm the presence of three separate sizes of inserts. All inserts were sequenced bidirectionally as shown in figure 5. Blastn alignment indicated that all clones corresponded to p54nrb or Non-O Non-POU domain protein. Genome blastn was not performed. This leaves the possibility that a better match still exists for chromosome 16 or that the library screen pulled only the more abundant sequences due to a slightly lower hybridization temperature.

Key Research Accomplishments

- Analyzed RACE reaction products for sequence content and compared the sequence to GenBank using NCBI blast protocols.
- Screened human prostate cancer cell lines for expression of nmt55 protein from nuclear extracts of LNCaP, C4-2 and PC-3 cell lines using nmt-1, nmt-4 and nmt-5 antibodies in western blot format. Nmt55 nuclear protein expression increases with progression when LNCaP compared to C4-2. PC-3 levels are similar to LNCaP (Aim 2). These data are the final demonstration that UG311 is not nmt55.
- C4-2 lambda phage library screened through 2 rounds for plaques hybridizing to UG311, UG101, UG102, UG307 (Aim 1a). Plasmids corresponding to the human UG311 (hUG311-#) were rescued from the lambda phage and sequenced (UVA Biomolecular Core Facility). Sequences were blastn searched to NCBI database and found to most closely resemble NonO-Non-POU 54kDa protein. This protein is a slightly different mRNA from nmt55 but encodes the same protein. The nmt55 western blot data suggest that we have cloned the more abundant member of the family and not the true UG311.

Reportable Outcomes

- The development of the urogenital sinus library and the screening of 746 clones in the LNCaP progression model. Patent proceedings have been dropped in the last quarter concerning these cDNAs. The primary data are being re-run in the GenBank databases to update the tables and sequence homologies. Anticipate publication this year.
- Appendix 2. Gregory CW, Degeorges A and **Sikes RA***. The role of the IGF axis in the development and progression of prostate cancer. In Recent Research Developments in Cancer Volume 3, Part II, 3: 437-462, 2001, (Eds) Pandalai.S.G., Mukhtar H and Labrie F, Transworld Research Network, Kerala, India., **Corresponding Author.** Since IGFBP's and other IGF-axis members were found to be expressed in the UGS library and may be important in prostate development and prostate cancer progression, this chapter does tie in directly with the goals of this grant. Also, since this occupied a significant portion of my time, then acknowledgement of DOD support is appropriate.
- Appendix 3. Robert A. Sikes, Ph.D. Curriculum Vitae.

Conclusions

- Since the expression pattern of nmt55 is constant or increases with serum withdrawal and malignant progression and UG311 decreases with progression of prostate cancer cell lines, then it is likely that nmt55 and UG311 do not encode the same gene.
- This increases the likelihood that the UG311 paralog is a novel member of this type of single-stranded nucleic acid binding protein family.
- Our efforts to clone the paralog have been thwarted to date by significant homology of the insert to a more abundant gene. This cloning will be pursued aggressively by RACE and using higher stringency conditions for the Lambda screening. Additional avenues will include the use of NCBI resources to identify the sequence by virtual homology followed by appropriate synthesis of primers to the mRNA.

References

1. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, in *Current Protocols in Molecular Biology*. 1999, John Wiley and Sons, Inc.: New York.

Legends to Figures

Figure 1. A) RACE control reactions using LNCaP-derived cDNAs. TFR is the transferrin receptor control. Int. is an internal RACE reaction control. B) Sample experimental RACE results. LNCaP cDNAs were subjected to 5'- and 3'-RACE reactions using UG311 specific primers as the anchors. The complexity of the 5'-RACE reaction is indicative of either multiple family members, splice variants, or sub-optimal PCR conditions.

Figure 2. RACE band sequences. Labeled as per clone identity. RACE fragments were gel purified and cloned into TOPO-TA vector for amplification and sequencing. Single-strand sequence was generated by the Biomolecular Core Facility at the University of Virginia using an ABI terminator-dye system and Sanger sequencing. A) Sequence derived from RACE bands. Sequence was then run through GenBank using the NCBI blastn algorithm. B) List of RACE fragments by name and corresponding BLAST alignment.

Figure 3. Western blots of 30 μg of KCl-extracted nuclear proteins. A) nmt-4 is a polyclonal antibody directed against the C-terminus of nmt55. B) nmt-5 is a polyclonal antibody directed against the N-terminus of nmt55. C) nmt-1 is a monoclonal antibody against the N-terminus nmt55.. Clearly by western blotting nmt-1 is the best reagent. As we started with nmt-4 it was necessary to continue with nmt-5 and nmt-1 respectively. The order was essentially chosen due to the amount of reagent available.

Figure 4. Analysis of phage inserts in rescued plasmid vectors. A) Gel of mini-prep DNAs of hUG311-#1-8. At least 2 distinct insert sizes are possible. B) Restriction mapping of the hUG311 inserts. Two micrograms of plasmid DNA was digested with BamHI and NotI to liberate the insert. Clones 2, 3and 7 have inserts of about 1 kbp. Clones 4, 6 and 8 have insert sizes of about 2 kbp. Clone 5 has an insert size of about 1.4 kbp. All of these inserts are smaller than the initial RNA blot indication for UG311 in human prostate cancer cell lines of 3.2 kbp. All inserts are smaller than the mRNA described for most of the NONO-like mRNAs. All plasmids were sequenced bidirectionally and compared to Genbank using NCBI Blast search (sequence and alignments not shown).

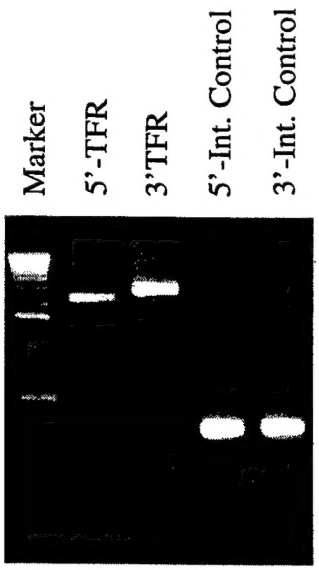
Appendices

1. Figures 1, 2, 3, 4

2. Gregory CW, Degeorges A and **Sikes RA***. The role of the IGF axis in the development and progression of prostate cancer. In Recent Research Developments in Cancer Volume 3, Part II, 3: 437-462, 2001, (Eds) Pandalai.S.G., Mukhtar H and Labrie F, Transworld Research Network, Kerala, India., **Corresponding Author**

3. Curriculum Vitae: Robert A. Sikes, Ph.D.

A.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3'TFR
5'-Int. Control
3'-Int. Control

B.

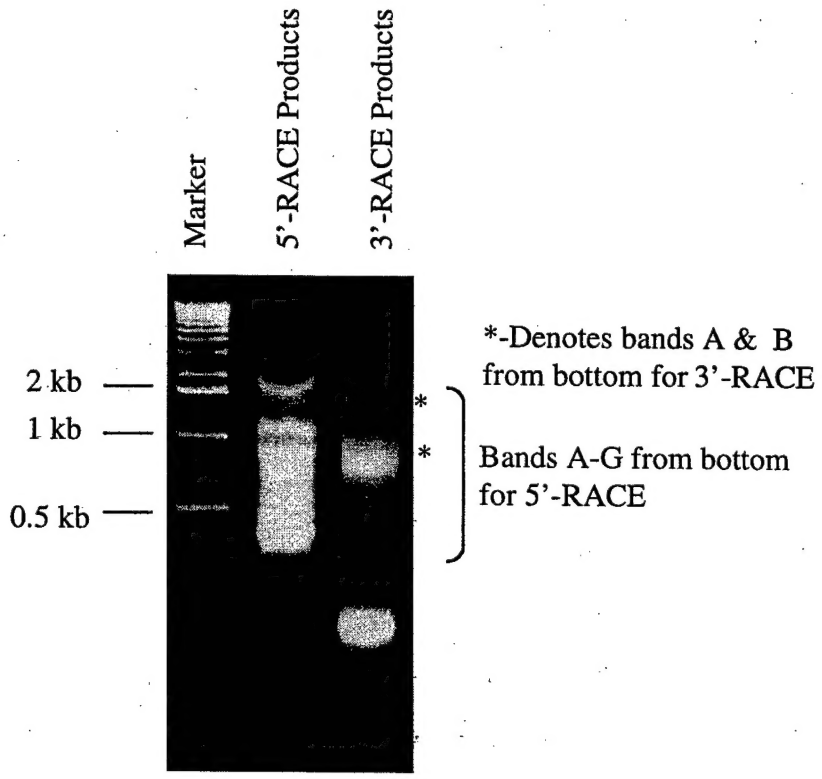
Marker
5'-RACE Products
3'-RACE Products
2 kb
1 kb
0.5 kb
*
*
*-Denotes bands A & B from bottom for 3'-RACE
Bands A-G from bottom for 5'-RACE

FIGURE 1

Figure 2A. Sequence obtained from UG311 5' and 3' RACE Reactions.

UG311 LN-9 01-7507 1 sln311 9 M13 R..

NANGNAAGAAANAGGANNANAANGNANGNANGANACGAACGAGAAGNAGNGANNCNNNAAGGNGGAGGNNNGNNNNNNNNNNNTNGGAG
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UG311 LN-30 01-7508 2 sln311 30 M13 R..

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UG311 LN-33 01-7509 3 sln311 33 M13 R..

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UG311 LN-8 01-7510 4 sln311 8 M13 R..

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TAGCAACTNATNACATGNTCTNCTNCANAATNNNCNNTAANNCACTACNTANNCACAATANTCNAANANCNCTACTNTNNAGACTACCN
TATANTNCNTANANATCNCANCTGACTCATANTANATCAATATTAATCTCACNTNACACTTANCTCANTNTNAANG

UG311 LN-3 01-7512 6 sln311 3 M13 R..
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NCTACTNCTTNCCCACNTNNNTCNNTNTGCTCTAAATNNNNTTNAAATTTTTCCTNCTTNNTTNTTTTATCTTNCCCCCCNNNCTNTTG
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ATATCTTACCACTNNNTTCCTTCNTTCNNCCTCCGATANCTTGCNCTCCACNANCCNCNCTACATNCTANTCNNTNNTATCCTTCNTCC
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GTCTCGTCNAANCTCTTNCTANTCCNCCCANAACANTTNTGCGATCTNTACTNCATCCTTATCCCNTCTCCNCCCCTCNTNNCTCTCTT
TNCACTCTTTNCTCAGTNNCCCNNATTTTNNTNTCTCCACTGNTTNNCAAATANTCTCNTCNTCCCACAGCTCCNTATNNTNTCACTCC
G

UG311 LN-11 01-7513 7 sln311 11 M13 R..
ANGGGNCNTCTCNACGNCNCNCCAAANCNTAANCNTNCTNCNGGCCANNCCTCTANCCTCTCNTCCNCNNANACACAGACAGAGGNGGC
NAAGAGGGNGGGTGACGACANGTTTTGAAACCCCCTTGGAAGGCCNCNCTNTNNNNNCCANNANANAACGCCNTNGGACNTACGGNNNA
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GNCNCCNGCGCNNCNGCCNNNCGNTACNNNTCGCNNNCACAACNTNCACNANNACTGNNTGACAAACACGTCACNCNCNGGATGAGANN
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ANNCNCAGNCGGANCNNANGNCTACNCNNNCANNCAATCCNCCCTCCGAGGGTGCANCNCACNCATACACGANACACGAGNCTCCNNGN
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UG311 LN-_3 01-7514 8 sln311_3 M13 R..

AANCANNTCCGCCANGGCNNTTCACGAGGNCNNCTAACACNNGGTNGNCCTATNTTNCCNAGCTAGNGNNANCNGGNGGGGTNTANGCN
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CNGCGNATNAGGAAACNATGTNCCNAGATNAGGANAGANAGGGGNANNNACNTCGNNCCTANNGTGGNTGNNATNNCNNNACCNGCNAG
NNNANNNNAGCNNNACAGNNGCGACANACNNNTNNCAACCGAGNGTAGNCANANTNGAANCNNNCNCGNNNANNNNANAGCTGAGGNGG
CGTANNGCNATANNACGCNGNCGGCGATATNTTGANANGAGNGNNACGCAAAAANGGGCNGNGACNGGATNNANAACCGAGCCGGGGCA
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NAGGCGNAGGNNCGNTANNNNGNAAAGAGANGACNANCGANGAGNGCAGACNTGNGAGTANNCCAACNATCGNGAANNANTANNTAGTN
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AGCGNANCTGATGNANAGNCGANCACGACNGTANNNNNCGNGT

UG311 LN-19 01-7515 9 sln311 19 M13 R..

NTNGCNACCNCACNANCNNTACNACTNNNACCCNCATTTGACCACCNTGATCNAANGCGCTNCCNCCTCNAGNTAGAGGNAGNAANNNG
CGNGGNNGAGNAGTNATTGGAAATCCCTTGNGAAACCCCNGAANANTNTAANGNAGGGACNNGACCGATCNCGGNGANNGACNANAAAA
AAAGANGCACNNGGNNCNAACNNTGTANANCCGCGGNNTNCACNAGCNTNATCGGNNTANGGGGGGNCNATNGGGGGGCCTNGAGATAA
NGGGAGNNNGNNCGAAANNATAACATAANANAANGGGTGCNTANACAATANCNNGNTCGNNCGGNNGANAGANNNGNANCNGCNGGAGA
NATAANCAACAGNNNGGAANANCNGNANANCCACANGANTCTCANNNCAAGGNNCNNGNCGNCGAGNCACAAANCACGATACGCNNNNN
CNNNNCNAACNNCTCGGGANANATNNANAATNCTCNCGAATGTNNNNNACGAATNCNGGCGGNCNCNAANANGACTNGCNNGGGAACAN
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GCGCNGTGNNGCCCCAGCNNCGGNCGGCGAGNNNCTGNCCGAGCGNNNCNAAACCGANNNNTNNNANNGCGANNGANAACNNAAANAGA
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CNGTATGCNNTGNGGGNTNNACNCNCTNANNGGTNGTGACNTNNNACAGTNNCGAGCNNGGNNGGNGANANTNANANCGNNNNACNANN
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GAGCCGCNGNCACGAGNCTAAATNNCTACTNTNGNCGTGGCNNGANNCAATNACNCGNNATNTCCGCGTNGNGNGGCGCGTGCNCANAA
NTAAGAGACGCGACGNNCCGCGNCTCNCGAACNCACACCCCACGCACNAGCNCGGCGTACACGCNGGNAGACAATNGACNACNTNCTNN
GCGTNGCNTANNACGT

Figure 2B. GenBank BLASTn homologies of RACE clones.

LN-9 Short homology to NaV1.1

gi|11597116|gb|AC010127.12|AC010127 Homo sapiens BAC clone RP11-2I8 from 2, complete sequence
Length = 190066

Score = 40.2 bits (20), Expect = 3.7
Identities = 20/20 (100%)
Strand = Plus / Minus

```
Query: 407    aaaagaaagaagagagggtg 426
              ||||||||||||||||||||
Sbjct: 127504 aaaagaaagaagagagggtg 127485
```

LN-30 HLA-A

gi|6469379|emb|AJ223972.3|HSAJ3972 Homo sapiens HLA-A gene, A*6808 allele
Length = 3187

Score = 78.0 bits (39), Expect = 1e-11
Identities = 45/48 (93%)
Strand = Plus / Minus

```
Query: 27   cgagctcggatcnctagtaacggncgccagtgtgctggaattcngctt 74
             |||||||||||| |||||||||| |||||||||||||||||| ||||
Sbjct: 3178 cgagctcggatcactagtaacggccgccagtgtgctggaattcggctt 3131
```

LN-33 ITGB4 gene for integrin beta 4 subunit, exons 3-41

gi|6453350|emb|Y11107.3|HSA011107 Homo sapiens ITGB4 gene for integrin beta 4 subunit, exons 3-41
Length = 28798

Score = 97.9 bits (49), Expect = 1e-17
Identities = 58/60 (96%), Gaps = 1/60 (1%)
Strand = Plus / Minus

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Query: 19    gctnggtaccgagctcggatcc-ctagtaacggccgccagtgtgctggaattcggcttct 77
              ||| |||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct: 17627 gcttggtaccgagctcggatccactagtaacggccgccagtgtgctggaattcggcttct
17568
```

LN-8 No Match

LN-12 No Match

LN-3 Human DNA sequence from clone RP11-10N16 on chromosome 1

gi|18476679|emb|AL590683.16| Human DNA sequence from clone RP11-10N16 on chromosome 1, complete sequence [Homo sapiens]
Length = 164684

Score = 74.0 bits (37), Expect = 2e-10
Identities = 57/64 (89%), Gaps = 1/64 (1%)
Strand = Plus / Minus

```
Query: 99     gtggcacaggtctgtaatctcanctactctgnaggctgangcactgggaattncttgaat
158
              ||||||||||||||||||||| |||||||| ||||||| ||| || ||||| |||||||
Sbjct: 109952 gtggcacaggtctgtaatctcagctactctggaggctgaggca-tgagaattgcttgaat
109894


Query: 159    ntgg 162
               |||
Sbjct: 109893 ctgg 109890
```

LN-11 Homo sapiens prostein protein (LOC85414), mRNA

gi|14916436|ref|NM_033102.1| Homo sapiens prostein protein (LOC85414), mRNA
Length = 3410

Score = 40.2 bits (20), Expect = 3.8
Identities = 20/20 (100%)
Strand = Plus / Minus

```
Query: 122  tgaaaccccccttggaaggcc 141
             ||||||||||||||||||||
Sbjct: 2186 tgaaaccccccttggaaggcc 2167
```

LN_-3 No Match

LN-19 No Match

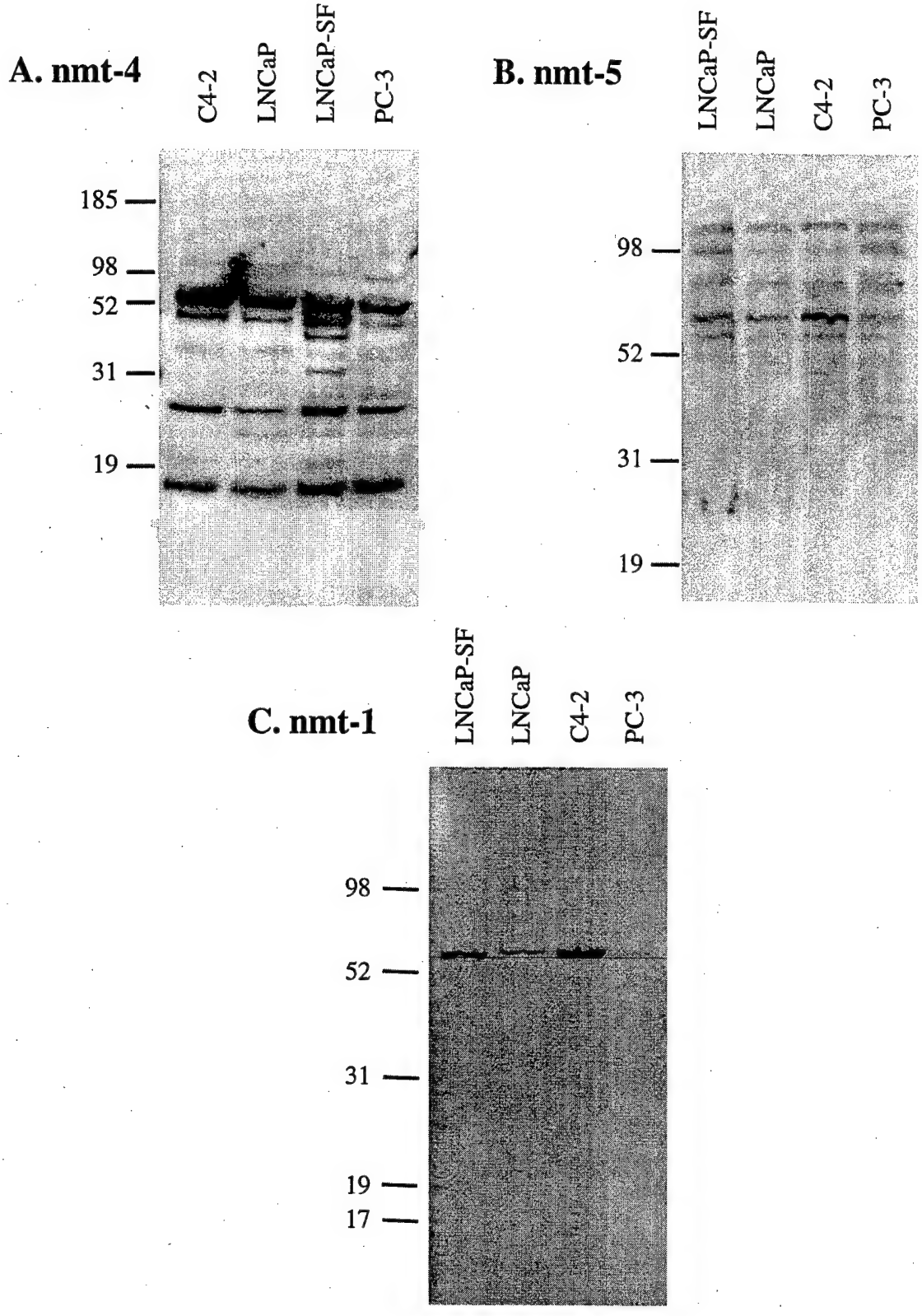
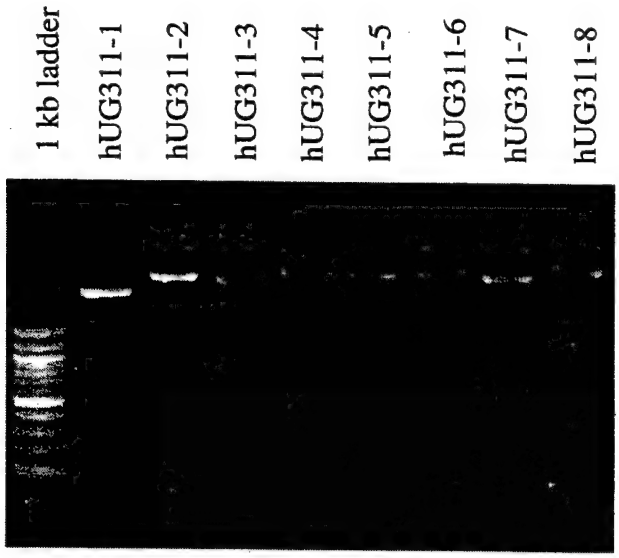

A. nmt-4
C4-2
LNCaP
LNCaP-SF
PC-3
185
98
52
31
19
B. nmt-5
LNCaP-SF
LNCaP
C4-2
PC-3
98
52
31
19
C. nmt-1
LNCaP-SF
LNCaP
C4-2
PC-3
98
52
31
19
17

FIGURE 3

A.


1 kb ladder
hUG311-1
hUG311-2
hUG311-3
hUG311-4
hUG311-5
hUG311-6
hUG311-7
hUG311-8

B.

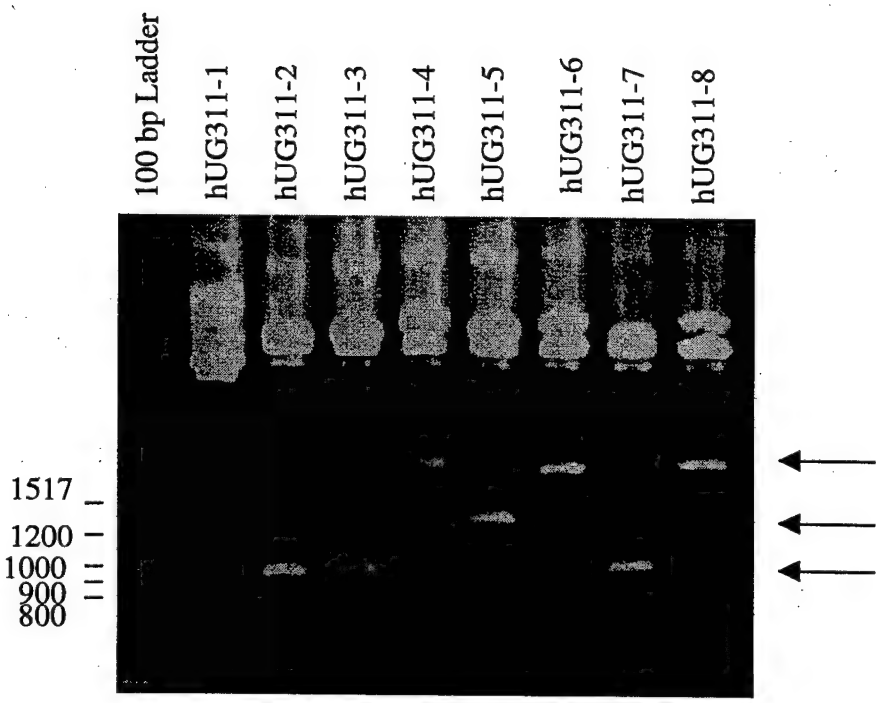

100 bp Ladder
hUG311-1
hUG311-2
hUG311-3
hUG311-4
hUG311-5
hUG311-6
hUG311-7
hUG311-8
1517
1200
1000
900
800

FIGURE 4

Appendix 2

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The IGF axis in the development and progression of prostate cancer

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ABSTRACT

Worldwide, prostate cancer is the third most commonly diagnosed cancer in men and the sixth most common cancer related mortality [1]. In North America prostate cancer is the most frequently diagnosed cancer and remains second only to lung cancer as the leading cause of cancer mortality [2]. The overall lifetime risk of developing an invasive prostate cancer is 1 in 6 for males in North America. With an estimated 396,000 cases worldwide and a 41.6% mortality rate, the impact of prostate cancer on the resources of health care is dramatic. Despite increased awareness and the widespread use in North America of the prostate-specific antigen (PSA) blood test, the incidence and mortality rates for prostate cancer have not changed significantly. Therefore, prostate cancer research strives to understand the mechanism(s) behind the development and progression of prostate cancer. As prostate cancer grows and progresses from localized to metastatic disease, there are changes in cellular adhesion and extracellular matrix molecules [3-10], motility [7-9, 11], responsiveness to androgenic steroids [6, 12-14], as well as dysregulated expression and/or response to peptide growth factors (GFs) or by GF receptors [15-17]. A better understanding of the changes in these systems will provide the basis for the design of new

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and more effective therapies to treat prostate cancer. The goal of this chapter is to provide an update on the role of the insulin-like growth factor axis in the development and progression of prostate cancer.

II. INTRODUCTION

Overview

The insulin-like growth factor (IGF) axis is comprised of two ligands (IGF-1 and IGF-2), the insulin-like growth factor receptors type I and type II (IGF1R and IGF2R, respectively), and six high affinity IGF binding proteins (IGFBP-1 to -6) [18-20]. On the surface, this appears to be a simple and straightforward system. The reality, however, is quite different. The action of the IGFs on various target tissues is mediated through systemic endocrine control of IGF-1 production by the liver. Target tissues also produce their own IGF-1 and -2. Furthermore, the effects of IGF in target tissues depend upon the availability and number of IGF receptors as well as the local production of the IGFBPs, which often varies by number and type within each target tissue. Ultimately, the net effect of the IGFs is determined by the interaction of the IGFBPs with the extracellular matrix and by the local production and action of IGFBP proteases.

Ligands

The ligands for the IGF axis are IGF-1 and IGF-2, which are 67 and 70 amino acids, respectively. They play a role in several critical biologic processes: apoptosis, cell cycle progression, differentiation, and regulation of gene expression [19]. IGF2 is important generally for fetal development whereas IGF-1 is dominant after birth. IGF is thought to mediate most of the effects of growth hormone [21-23]. This being said, rodents downregulate IGF-2 expression in adults while humans continue to express both ligands through adulthood [24, 25]. The crucial role for the IGF axis in development was demonstrated by genetic knockout of different members of the IGF axis, either alone or in combination, in transgenic mice [23, 26-28]. Single deletions of either IGF-1 or IGF-2 resulted in a marked reduction in postnatal survival and growth deficiency of offspring to only 60% of control. Double knockouts resulted in neonatal lethality and further exacerbation of the dwarfism to about 30% of control size [28]. By contrast, the overexpression of GH or IGF-1 in transgenic animals resulted in dramatic overgrowth of somatic tissues, but these studies also demonstrate that GH and IGF-1 have independent actions [29-32]. With the exception of the testes, kidneys, and adrenal glands, IGF-2 overexpression did not result in similar overgrowth of somatic tissues in transgenic animals [33]. The binding affinities of IGF-1 and IGF-2 to the IGF1R are quite similar at 1 nM and 3 nM, respectively. The final interaction and subsequent action of the IGFs is determined by the association of the IGFs with particular binding proteins (see below). Furthermore, the half-life of both IGFs is dramatically extended when complexed with IGF binding proteins [19, 20].

Receptors

There are at least three closely related receptor tyrosine kinases in the insulin receptor family. These are the insulin receptor (IR), the insulin related receptor (IRR), and the IGF1R [18, 34, 35]. Knockout mice experiments have genetically identified a fourth, highly specific, temporally restricted receptor for IGF-2 in development, which is called IGFRX due to an overall lack of understanding for the structure, signaling, etcetera, of this receptor [26, 28]. The IR, IRR and IGF1R share several features. They are all $\alpha_2\beta_2$ heterotetrameric transmembrane glycoproteins where the α and β subunits are synthesized from a single mRNA as a large precursor. This precursor is then glycoslyated, proteolytically cleaved, and crosslinked by cysteine bonds to yield the mature $\alpha\beta$ chain. The α-chains are entirely extracellular while the membrane spanning ß –

chains are responsible for intracellular signal transduction in response to ligand. The mature IGF1R has an apparent molecular weight of 320 kDa. There are two high affinity ligands for the IGF1R, IGF-1 (1nM) and IGF-2 (3nM), that upon binding to the α-chains induce the tyrosine autophosphorylation of the IGF1R ß-chains. This receptor autophosphorylation initiates a cascade of signal transduction that results in either growth, survival, or differentiation of the target cell [18, 19]. While the molecules involved in the IGF/IGF1R signal transduction cascade are somewhat cell-type specific, there are some common themes regarding proliferation versus apoptosis/ survival. More importantly, IGF signaling has been shown to engender a survival advantage to cells subjected to a variety of potentially lethal insults that include UV and X-irradiation, chemotherapy, oxidative stress, loss survival factors and loss of adhesion [36-48]. The affinity of insulin for the IGF1R is about 100 times lower and *vice versa*. Therefore, the insulin receptor is only activated at supraphysiological concentrations of IGFs and *vice versa*. The ligand for the IRR has not been identified to date.

The IGF2R, also known as the mannose-6-phosphate receptor, is a 270 kDa glycoprotein that has no homology to the insulin receptor tyrosine kinases. The IGF2R is a bifunctional protein that binds lysozomal enzymes bearing the mannose-6-phosphate recognition site and IGF-2 at different binding sites [49]. To date, no intracellular cell signaling has been found from the IGF2R, suggesting that the IGF2R facilitates the degradation of IGF-2 by transporting IGF-2 to lysozomes, thereby limiting the signal potentiated by the growth factor. Alternatively, the IGF2R may act as a slow release mechanism for excess IGF-2. Indeed, knockout mice for the IGF2R have a 135% increase in body weight compared to control littermates. The importance of the IGF2R is also illustrated by these studies. IGF2R knockouts have numerous developmental abnormalities and usually die perinatally [27, 50].

Binding Proteins

IGFs are transported in plasma and extracellular spaces by high affinity binding proteins. These IGF binding proteins (IGFBPs) range in molecular weight from 25 to 40 kDa [19, 20, 51, 52]. More than 80% of IGFs in circulation are bound to IGFBP-3 in the presence of an 88 kDa non-IGF binding acid labile subunit (ALS) to form a 140 kDa ternary complex. Six high affinity IGF binding proteins (IGFBPs) have been cloned and isolated. These binding proteins are remarkably well conserved. They share at least 50% homology with each other and more than 80% homology between species [53, 54]. Furthermore, with the exception of IGFBP-6 that preferentially binds IGF-2, the other IGFBPs bind both IGFs with affinities comparable or superior to the receptors. Despite these similarities, many characteristics are different: chromosomal location, RGD recognition site, preference for binding IGF-1 or -2, as well as glycosylation and phosphorylation differences. IGFs bound to IGFBPs represent the reserve pools of IGF. In this context, several major functions are suggested for the IGFBPs [19, 20] : (1) to act as transport proteins in plasma and to control the efflux of IGFs from vascular space; (2) to prolong the half-lives of the IGFs; (3) to provide a means of tissue- and cell- type specific localization; (4) to modulate directly the interaction of the IGFs with their receptors and thereby indirectly control biological actions; (5) to assist in the regulation of blood glucose levels and (6) ligand independent effects of IGFBPs, most notably IGFBP-3, that are mediated through receptors that are distinct from the IGF receptors.

The remainder of this review will discuss recent insights on the role of the IGF axis in prostate neoplasia. The IGF system takes into account most of the complexities observed in prostate pathologies. As discussed briefly above, the IGF axis comprises a complex

system of ligands, receptors, binding proteins, and proteases that interact with the extracellular matrix and respond as well as modulate the host endocrine milieu. These characteristics make the IGF axis a valuable and interesting domain for study.

III. THE PROSTATE AND THE IGF AXIS

A. Ligands and Receptors.

IGF-1, IGFBP's and Prostate Cancer Risk

The role of the IGF axis in prostate carcinogenesis is becoming increasingly emphasized. Recent studies show that elevated levels of plasma IGF-1 predict an increased risk of up to six-fold for prostate cancer [55-58]. While a statistical association to prostate cancer is evident there is no direct evidence that elevated serum IGF-1 causes the initiation of prostate cancer. Recent reviews, [57, 58]], interpret the data on elevated IGF levels with the following conclusions. First, IGFs, IGFRs and IGFBPs are expressed in prostatic tissue and IGFs promote growth, while IGFBPs largely inhibit growth. Second, IGFBP-3 has a pro-apoptotic effect in addition to its inhibitory effect on IGF-1 in the prostate. This data has support in the reports by Chan et al.[55] and Hankinson et al. [59] which found a protective effect of elevated IGFBP-3 in patient sera. Third, other binding proteins may be better measures of having a prostate cancer. IGFBP-2 was measured and found to be up to three-fold higher in the serum of prostate cancer patients as compared to normal controls [57, 60-62]. Finally, that elevated serum IGF may contribute to the development of benign prostatic growth, or hyperplasia (BPH), and not to prostate cancer. These conclusions come from studies of growth hormone replacement therapy where no increased risk of prostate cancer was found [57]. That said, in acromegaly, a condition characterized by chronically elevated GH/IGF-1, there is dramatic association with the incidence of BPH but not prostate cancer [63-65]. Therefore, an elevated level of serum IGF-1 could arise from several sources, including a prostate tumor, and may or may not contribute to the initiation of prostate cancer. It is currently unclear whether or not there is a benefit to be gained using measurements of IGF-1, IGFBP-2 or -3 to predict risk or to aid in the diagnosis of prostate cancer. Finally, the IGF responsiveness of a prostate cancer will be determined by the interaction of the available serum IGF-1 and the expressed milieu of IGF axis components in the prostate, as discussed below.

The IGF axis and Androgens

The maintenance of structure and function in the prostate depends upon the presence of androgens [66] . Furthermore, most metastatic prostate cancer responds favorably to castration [67-69]. However, as prostate cancer progresses it becomes more androgen independent [66, 68-70]. Therefore, another issue concerning prostate cancer is the regulation of the IGF axis by androgens. There are few publications in this area of inquiry. An in vitro study using LNCaP cells showed that very low concentrations of DHT synergized with IGF-1 using thymidine uptake as a readout, while IGF-1 alone had only modest response [71]. In androgen responsive foreskin fibroblasts, IGFBP-3 was not regulated by either IGF-1 or testosterone, but IGFBP-5 was upregulated by IGF and increased synergistically by IGF and testosterone in combination [72]. Interestingly, IGFBP-4 in foreskin fibroblasts was completely degraded in response to IGF-1, while testosterone had no effect on the expression or degradation with or without IGF-1. Goosens et al.,[73] showed that the mRNAs for IGFBP-2 and -4 were increased, while IGFBP-3 was repressed in LNCaP cells treated with androgen. PC-3 cells transfected with androgen receptor resulted in a decreased steady-state level of IGFBP-3 protein and mRNA. The transfected PC-3 cells also have decreased cell proliferation rate [74]. The expression of stromal IGF-1 or IGF-2 might be modulated by an androgen-induced peptide, IGFBP-3. In the

Dunning rat model, the expression of IGF-1 was induced by androgen in androgen-dependent but not in androgen-independent tumors maintained in castrated hosts [75]. The patterns of expression of the mRNAs were different, depending on the degree of malignancy. In the ALVA prostate cancer cell line, derived from surgical specimens of adenocarcinoma of the prostate, the levels of IGFBP-4 and IGFBP-6 were not modified by androgens [76]. In the androgen-dependent CWR22 prostate cancer xenografts, IGFBP-5 was the only IGFBP regulated by androgens [77]. In the androgen responsive Shinogi tumor model, castration induced rapid increases in IGFBP-5 mRNA and decreases in IGFBP-3 and -4 mRNAs and no effect on IGFBP-2 mRNA levels [78]. The expression level of IGFBP-2, -3, -4 and -5 mRNAs is increased after castration and involution of the rat ventral prostate [79]. These data show that most IGFBPs are androgen repressed in vivo. This study also showed that IGF1R mRNA expression is dependent upon androgen for expression and is lost rapidly after castration. Huynh et al. [80] used the 5α-reductase inhibitor finasteride to demonstrate the dependence of IGF-1 and IGF1R expression on the presence of 5α-dihydrotestosterone, the active male steroid in prostate tissue, and not simply testosterone. This study also showed an increase in IGFBP-3, further confirming its suppression by androgen in prostatic tissue. Similarly, the use of finasteride demonstrated that IGFBP-5 suppression was due to 5α-dihydrotestosterone [81]. These findings have now been extended to humans [82]. Men taking finasteride also demonstrated an increase in IGFBP-2, -4 and -5, as well as a decrease in tissue IGF-1. Interestingly, only IGFBP-2 and -4 co-associated with apoptotic markers. All these examples demonstrate a close regulation of the IGF axis by androgens and an involvement of this axis in the evolution towards androgen independence. However, the cell context does appear to determine the ultimate regulation of the IGF axis by androgens and IGF.

IGFs and IGFRs in the Prostate

Pietrzkowski et al. demonstrated the existence of an autocrine loop of proliferation involving IGF-1 and the IGF1R in both androgen-independent human prostate cancer cell lines, DU145 and PC3, as well as in androgen-sensitive cell line, LNCaP [83, 84]. However, these results on the expression of IGFs in prostate epithelial cells are controversial. Subsequent studies could not demonstrate the secretion of IGF-1 by the same prostate cancer cell lines [71, 85, 86] and only very low levels of IGF-1 mRNA in LNCaP [86]. These findings do not completely abrogate the early reports by Pietrzkowski, since an autocrine proliferation loop was found in PC3 [87], DU145 [85] and LNCaP cells [86]; however, it is likely due to the expression of IGF-2 as the ligand (See Table 1 for Summary). Furthermore, it is clear that prostate cancer cell lines respond mitogenically to exogenous IGF-1 and bind IGF-1 with high affinity, thereby indicating the presence of the receptor [71, 88]. There have since been several studies [73, 86, 88] (Sikes et al. unpublished observations) to demonstrate the expression of both IGF1R mRNA and protein in prostate cancer cell lines.

Similar studies to determine IGF axis member expression were performed on primary cultures of epithelial and stromal cells derived from normal prostate, BPH, and cancer (Table 1). Epithelial cells and stromal cells express the type I receptor for IGF but not the type II [89, 90]. Normal epithelial cells are sensitive to the mitogenic effect of IGF-1 and IGF-2, but they do not express either ligand [91]. Stromal cells, specifically BPH [40, 91] and cancer [91] stromal cells, produce large amounts of IGF-2 . Another study showed that under different experimental conditions, both epithelial and stromal cells express IGF-2 [92].

There are now several studies that have

Table 1. Expression of IGF axis members in prostate cancer cell lines and primary cultures.

	Cell Lines					Normal[1]		BPH[1]	Cancer[1]
	LNCaP	C4-2[a]	Du145	PC-3	P69[f]	Ep.[g]	Stroma[i]	Stroma	Stroma[i]
IGF-1	nd[a,c,d], +/-[b]	nd	nd[a], -[b]	-[b,c], nd[e]	-	nd	nd	+/-[h]	nd
IGF-2	+[a], +/-[b], nd[c,d]	++	nd[a], ++[b]	+[e], ++[b],nd[c]	+	nd	+	+++[h], ↑10x[i]	+
IGF1R	+[b,c],++[a], nd[d]	+++	+[a,b]	+[b,c,e]	+	+	nd	+[h]	nd
IGF2R	+[a], ++[b], nd[c,d]	++	nd[a], ++[b]	++[b], nd[c,e]	nd	-	nd	+[h]	nd
IGFBP-1	nd[a,b,c], -[d]	nd	nd[a,b]	nd[b], -[c,e]	-	-	-	nd[h]	-
IGFBP-2	+[a,b,c], ++[d]	+	+[a,b]	+[b,d], -[c]	+	+	+	nd[h], -[l]	+
IGFBP-3	+/-[a], -[b], +[c,d]	+	+[a,b]	+[b], +/-[c], ++[e]	+	-	+	nd[h], var.[l]	+++
IGFBP-4	inc[a], +/-[b,d], -[c]	inc	inc[a], +[b]	+[b,c,e]	-	+	var.	nd[h]	var.
IGFBP-5	+++[a], -[b,d], +[c]	+/-	+[a], -[b]	+[b,c], -[e]	+	-	+	nd[h],↑4x[i]	+
IGFBP-6	nd[a,c], -[b,d]	nd	nd[a],+[b]	++[b], -[c], +[e]	+	-	-	nd[h], -[i]	-

[a]Sikes et al., FACS, IHC, WLB, RT-PCR (unpublished results); [b]Kimura et al., RNA blot, RT-PCR IHC (J. Urol, 3:39, 1996); [c]Conover et al, WLB, WB (JCEM 80:987, 1995); [d]Goosens et al. RNA blot. (Mol. Cell. Endocrinol. 155: 9, 1999); [e]Angelloz-Nicoud and Binoux, WB, WLB, RIA (Endocronol. 136: 5485, 1995); [f]Plymate et al., RNA blot/protection (JCEM 81: 3709, 1996); [g]Cohen et al., WLB, RNA blot (JCEM 73:401, 1991); [h]Grant et al., RT-PCR (JCEM 83:3252, 1998); [i]Cohen et al., WLB, RNA blot (JCEM 79:1410, 1994)

[1]Primary Cultures from histologically normal tissues, benign prostatic hyperplasia or prostate cancer. nd = not determined; inc = inconclusive due to antibody cross-reactivity; var= variable expression

looked at the expression of IGF axis members in human tissues (Table 2). Figueroa et al. [93], used RNAse protection to show the expression of IGF-2 and IGF1R. Tennant et al. PIN [94-96] used in situ hybridization and immunohistochemistry on tissues to examine the expression of IGFs, IGFRs and binding proteins in prostate cancer samples, BPH and high grade PIN. These studies demonstrated that IGF-2 is expressed by both stromal and epithelial cells and that the message for this growth factor is increased in prostate cancer. The IGF1R was expressed by both stromal and epithelial cells, but the level was decreased in adenocarcinoma compared to benign epithelium [95]. These results are in contrast with other neoplasia where autocrine proliferation loops often lead to increased expression of the receptor. A partial explanation can be found in the control of IGF-2 expression. The IGF-2 gene demonstrates paternal allele-specific expression, paternal imprinting, and is found in most normal adult and fetal tissues [22]. However, a loss of imprinting for the IGF-2 gene was demonstrated in normal prostate and shown to persist in prostate tumors [92]. A relaxation of this imprinting might explain the overexpression of IGF-2 and the initiation of autocrine stimulation observed in prostate cancer.

Table 2. Reported expression and localization of IGF axis members in prostate tissues.

	Prostate Tissues				Cellular Localization	
	Normal	BPH	PIN	CaP	RNA	Protein
IGF-1	nd	+[e]	nd	nd	(S>>E)	nd
IGF-2[b,d]	+	+	↑ wk	↑↑30%*	(E>S)	(E=S)
IGF1R[b]	+	+	↓42%*	↓35%*	(E>>S)	(E>>S)
IGFBP-1[a]	-	-	-	-	-	nd
IGFBP-2[a,c]	+	+	+[a], ↑[c]	↑↑* (GS7-10)	+[a]	nd[a], ↑E[c]
IGFBP-3[a]	+	+	+	↓↓* (GS7-10)	+[a]	nd[a], ↑E[c]
IGFBP-4	+[a,b]	+[a,b,e,f]	nc[a],↑[b]	↑↑*[b] ,nc[a,f]	(E>S)[b,e]	(B+E>S wk)[b]
IGFBP-5[a,b]	+	+	↑wk	↑↑*(GS7-10)	(S**>>E)[b]	(E>B>S mod)[b]
IGFBP-6[a,b]	++	++[f]	++	+[f]	(E>S)[b]	(E>B>S wk)[b]

[a]Figueroa et al, RNase protection. (J. Urol. 159:1379, 1998.)
[b]Tennant et al, In Situ and IHC, (JCEM 81:3774 or 81:3783, 1996.)
[c]Thrasher et al, IHC, (J. Urol. 155:999, 1996.)
[d]Li et al, (Cell Tissue Res., 291:469 , 1998.)
[e]Barni et al., In Situ, RIA, RNA blot (JCEM 78: 778, 1994)
[f] Goosens et al. RNA blot. (Mol. Cell. Endocrinol. 155: 9, 1999)

S=stromal localization; E=epithelial cell localization; B=basal cell localization;
wk=weak or small change; nd=not determined; GS=gleason score
*statistically significant; **strong staining in periglandular fibroblasts

Finally, IGF-2 has at least two isoforms, a 15 kDa "big" IGF-2 and the more often described 7.5 kDa IGF-2 [97]. "Big" IGF-2 was shown to be more effective at stimulating tyrosine phosphorylation of the IGF1R than either IGF-1 or IGF-2. Furthermore, both IGF-2s were more mitogenic than IGF-1. Interestingly, Li et al. [98] showed that breast, prostate, and bladder cancers expressed the mRNA for the "big" IGF-2 isoform. Similar results were found in a rhadomyosarcoma model system where a 10 kDa form of IGF-2 was found [99]. The question remains to be addressed whether these modifications are a consequence of or are responsible for malignant transformation.

There is experimental evidence to support the critical involvement of the IGF axis in prostate cancer growth. Pollak et al. [100] showed a decrease in the growth rate of PC-3 xenografts in IGF-1 deficient mice. Antisense RNA to IGF1R has been shown to suppress tumor growth and prevent invasion by rat prostate cancer cells in vivo [101]. IGF-1 analogs that block IGF1R activation prevent prostate cancer cell growth [84]. Neutralizing antibodies to the IGF1R also prevent prostate cancer cell growth [86]. Therefore,

comprehension of the tight regulations and interactions of IGF axis will probably give new insights to the understanding of prostate cancer.

B. Binding proteins and proteases

IGFBP-1

Introduction

The human gene for IGFBP-1 is located on chromosome 7 [102] and encodes a 25 kDa protein that contains an RGD motif. The RGD motif is recognized as a determinant for integrin binding suggesting that ligand-independent effects of IGFBP-1 could be mediated through integrin heterodimers [103]. In Chinese hamster ovary cells IGFBP-1 stimulates migration that can be abolished by mutating the IGFBP-1 RGD motif [104]. IGFBP-1 could also be co-immunoprecipitated with antibodies to the $\alpha 5$ integrin subunit that cemented the involvement of IGFBP-1 in integrin mediated migration. IGF-1 had no effect on this activity. IGFBP-1 is expressed in the placenta [105] where it may limit trophoblast invasion. It is expressed in skeletal myoblasts, where it modulates differentiation into myotubes [106]. IGFBP-1 is also made by the liver, where its expression is suppressed by insulin and increased by glucagon [107]. IGFBP-1 is therefore tightly regulated by nutritional status [108] and is thought to regulate tightly the pool of free IGF-1 and its half-life in circulation [109]. Furthermore, IGFBP-1 is found in several phosphorylation states that determine its affinity for the IGFs [110, 111]. Phosphorylated IGFBP-1 has a six-fold higher binding affinity than the unmodified binding protein. Furthermore, it is the phosphorylated IGFBP-1 that has been implicated in growth inhibition. Interestingly, at this time the change in affinity for IGF has only been observed for the human IGFBP-1 and not for the rat.

IGFBP-1 in the prostate

IGFBP-1 has been used to demonstrate the growth inhibitory effects of IGFBPs on IGFs in prostate cancer cell growth and the existence of an autocrine proliferation loop [85]. However, there has been no description of IGFBP-1 expression in prostate tissues or cell lines to date [40, 73, 87, 89, 91, 93, 112]. Koutsilieris et al. [113] isolated a serine protease activity from the prostate tumor cell line PA-III that cleaves IGFBP-1 and -2. This activity increased the growth of osteoblastic and PA-III cells and could be blocked by antibodies to IGF-1. Therefore, while not directly made by the prostate or prostate cancer cells, IGFBP-1 may have a role in the regulation of prostate cancer behavior in most frequent site of prostate cancer metastasis, the bone.

IGFBP-2

Introduction

The gene for IGFBP-2 is located in chromosome 2 [102] and encodes a 31 kDa protein [19]. IGFBP-2, like IGFBP-1, contains an RGD motif, but no binding to integrin receptors has been documented to date. There are also no reports of IGFBP-2 being either glycosylated or phosphorylated. In the 13.5 day post-coital mouse, IGFBP-2 expression is localized to the lungs, liver, kidney, choroid plexus and floor plate [114]. In most cases the mRNA and protein co-localize; however, in the tubule of the kidney the mRNA for IGFBP-2 is in the epithelium and the protein is localized in the stroma. IGFBP-2 is increased in the follicular fluid from patients with polycystic ovary disease, suggesting a stimulatory role in this tissue [115]. IGFBP-2 is overexpressed in glioblastoma multiforme [116], leukemic T-cells [117], adrenocortical tumors [118], neuroectodermal tumors [119], and cell lines from breast cancer [120-122], Wilm's tumor [122], colon cancer [122],

Burkitt's lymphoma [122], and lung cancer [122, 123].

IGFBP-2 Modulation of IGF Action and Expression in Prostate

Transgenic animals overexpressing IGFBP-2 have markedly reduced body weight, suggesting a largely antagonistic role for IGFBP-2 in mediating the effects of the IGFs. Likewise, retinoic acid suppression of IGFBP-2 corresponds to increased mitogenicity in human lung alveolar cells [124]. This is clearly context dependent, as IGFBP-2 enhanced the mitogenic effects of IGF-1 in the MCF-7 breast cancer cell line [125]. Similarly, in estrogen-receptor negative breast cancer cell lines, where IGF-1 stimulated cell growth, IGFBP-2 was upregulated [121]. Also, many colon cancer cell lines express IGFBP-2 at some level [122]. Furthermore, overexpression of IGFBP-2 in an epidermoid carcinoma resulted in elevated levels of IGF, increased tumor take and growth [126]. More recently, increased IGFBP-2 levels were associated with the development of an androgen-independent phenotype in the Shionogi tumor model that further suggest a stimulatory role in cancer progression [78]. BPH is a benign proliferative disease of the prostate characterized by the overexpression of IGF-2 by stromal cells and a decreased expression of IGFBP-2 with a concomitant increase in IGFBP-5, which is not expressed in normal prostate (Table 1) [91]. These changes were presumed to regulate the bioavailability of IGFs in favor of growth stimulation, since IGFBP-2 had the tendency to inhibit IGF action in stromal cells, whereas IGFBP-5 stimulated IGF effects. Similarly, in neuroblastoma cells, IGFBP-2 appears to be inhibitory as retinoic acid (RA) treatment initiates rapid degradation of IGFBP-2 and an increased synthesis of IGF-2 [127]. As RA promotes cell growth both the proteolysis of IGFBP-2 and the increased IGF-2 synthesis would serve to increase the free pool of IGF.

IGFBP-2 was shown to have altered levels of expression in prostate pathologies (Table 2). In contrast to BPH, the level of IGFBP-2 in patient serums is increased in prostate cancer [61]. Tennant and colleagues [96] analyzed IGFBP expression at both the protein and mRNA levels in prostate tissues. They showed that both IGFBP-2 protein and mRNA were increased in PIN and increased further in adenocarcinomas as compared to benign tissue. This may reflect different mechanisms of action for IGFBP-2 in stromal versus epithelial cells in the prostate. Recently, cDNA expression arrays coupled with tissue microarray analysis have determined that IGFBP-2 is overexpressed in 100% of hormone-refractory prostate cancers, 36% of primary prostate cancers and none of the benign samples tested [128]. IGFBP-2 has the potential to associate with cell surface ligand (or recognition site) through a RGD sequence. Binding of IGFBP-2 to an integrin receptor may facilitate transport of IGF-1 to the type I IGF receptor or may stimulate cell motility as shown for IGFBP-1 [104]. IGFBP-2 was also shown to bind to heparan and ECM when complexed with IGF-1 or IGF-2 [129]. This is very interesting when one considers that many growth factors known to stimulate prostate epithelial cells are heparin binding, including some unknown factors produced by an osteosarcoma cell line [130].

Proteolysis of IGFBP-2

The degradation of IGFBP-2 has been associated with a decreased affinity for IGFs, thereby providing a mechanism for overcoming the growth inhibitory role of this binding protein [20]. In neuroblastoma the proliferative effects of bFGF have been attributed to the degradation of IGFBP-2 by a metalloproteinase [131]. Several groups have shown that Cathepsin D can mediate IGFBP proteolysis in prostate cancer cell lines [112, 132]. While this is largely considered a lysomal protease active at acidic pH, it has

been found in the conditioned media from several cancer cell lines. Furthermore, the surface of cancer cells appears to be highly acidic in nature and corresponds with metastatic potential in rat prostate cancer cells [133]. In the lung, matrix metalloproteinase 1 (MMP-1) is responsible for the degradation of IGFBP-2 [134]. Overexpression of this MMP probably plays a role in asthma. Koutsilieris et al. [113] showed that rat prostate cancer cells (PA-III) secrete urokinase plasminogen activator that degrades IGFBP-2 from osteoblasts. Other metastatic prostate cancer cell lines also secrete urokinase which has been associated with an increased metastatic potential [135-139]. Therefore, expression of IGFBP proteases like urokinase, MMPs, and kallikreins (PSA, hK2) may facilitate prostate cancer metastasis to bone.

Regulation of IGFBP-2 Expression

IGFBP-2 is positively regulated by IGF-1 and negatively regulated by retinoic acid (RA) in estrogen receptor negative breast cancer cell lines [121]. RA also decreases expression of IGFBP-2 in neuroblastoma, although the regulation is by proteolysis and decreased mRNA stability and not through protein synthesis, secretion, or transcriptional regulation [127, 140]. Castration induces the expression of IGFBP-2 in the rat [79], but this cannot be due to the action of the biologically active androgen 5α-DHT since the administration of finasteride cannot reproduce this effect [80]. In humans, however, finasteride administration does result in an upregulation of IGFBP-2 levels [82]. Goosens et al. [73] found an upregulation of IGFBP-2 mRNA in response to low dose androgen treatment in the LNCaP human prostate cancer cell line. It is not clear whether this is associated with an increase in IGFBP-2 protein expression or not. Hypoxia has been shown to upregulate the expression of IGFBP-2 and IGF1R in the retina [141]. Tumors ultimately outgrow their vascular support which leads to hypoxic conditions [142]. Many genes upregulated by hypoxia are targeted towards neovascularization, like vascular endothelial growth factor (VEGF) [142-146]. Interestingly, VEGF is also regulated by IGF-1 in colon cancer [147]. In the LNCaP progression model of human prostate cancer [148, 149] there is increasing VEGF production along with increasing IGF-2 as the cells become more androgen independent [150] (Sikes et al. Unpublished observations, Simons et al., Personal communication).

IGFBP-3

Introduction

The gene for human IGFBP-3 is located on chromosome 7 in close proximity to the gene for IGFBP-1 [102]. IGFBP-3 is a 45-50 kDa protein containing three N-linked glycosylation sites and a nuclear localization signal (NLS) sequence. IGFBP-3 is the major circulating IGFBP and the main IGF carrier in serum. Free IGFBP-3 has a high affinity for IGF-I and II and most of the serum IGF-I circulates as a 150 kDa complex with IGFBP-3 and an acid labile subunit [19]. This high affinity binding allows IGFBP-3 to sequester IGF-I, preventing IGF-I binding to its cognate receptor, thus blocking IGF-I action and causing IGFBP-3 to inhibit cell growth. Early studies showed that transfection of the IGFBP-3 gene into Balb/c fibroblasts inhibited cell growth [151] and that IGFBP-3 blocked IGF-I stimulated DNA synthesis in human skin fibroblasts [152]. Senescent fibroblasts were shown to overexpress IGFBP-3 [153, 154]. Cohen and colleagues demonstrated that IGFBP-3 induced apoptosis and mediated TGF-β1-induced apoptosis in prostate cancer PC-3 cells [155, 156]. Moreover, IGFBP-3 was shown to decrease proliferation and to modulate retinoic acid- and TGF-β2-induced growth inhibition in breast cancer cells [157, 158].

IGFBP-3 in Growth and apoptosis

IGFBP-3 was detected in conditioned medium from human prostate epithelial cells [159] and from prostate stromal cultures [90, 91] (Table 1). The androgen-independent prostate cancer cell line PC-3 was also shown to secrete IGFBP-3 in addition to IGFBP-2, -4, and -6 [87]. In PC-3 cells, proteolytic fragments of IGFBP-3 were prevalent. The authors suggested that the biological activity of IGF-I and -2, which are normally bound to IGFBP-3, may be directly related to the proteolysis of the binding protein. That is to say, increased IGFBP-3 proteolysis prevents it from binding to serum IGFs, thus allowing IGFs to activate signaling pathways in the cell to induce growth. In contrast, PC-3 cells stably transfected with a constitutively active androgen receptor did not secrete IGFBP-3. However, when treated with IGFBP-3/IGF-I in combination, the cells increased their proliferation rate, suggesting that higher concentrations of IGFBP-3 may result in an enhancement of the IGF-I response [74].

IGFBP-3 may be a direct inducer of apoptosis in both breast and prostate cancer, independent of IGF-I effects. Incubation of breast cancer cells with a non-glycosylated IGFBP-3 sensitized the cells to ceramide-induced apoptosis in an IGF-independent manner [160]. Similarly, IGFBP-3 caused apoptosis in prostate cancer PC-3 cells independent of the IGF-IGF receptor and p53 pathways [156]. IGFBP-3 was implicated as a serum component likely responsible for high-serum induced apoptosis in PC-3 cells [155].

Proteolysis of IGFBP-3

Proteolytic fragments have lower affinity for IGFs, thus allowing increased levels of free IGFs to bind to IGF-I receptors. Prostate-specific antigen was the first described IGFBP-3 protease [161]. The kallikrein-like serine proteases, the cathepsins [112] and the matrix metalloproteinases [162] all have been shown to cleave IGFBP-3. It is interesting to note that all three of these protease families are expressed in prostate and/or prostate cancer. Some of these proteases were not found in the carcinomatous elements but rather were located in stromal cells producing NGF [163] or other stromal components, as described in other organs [164]. Recently a direct role of MMP-9 was assessed in the autocrine proliferation of DU145 cells. The IGF-1 autocrine proliferation loop is regulated by IGFBP-3 whose activity may in turn be regulated by IGFBP-3 proteases, in particular MMP-9 [162]. Decreasing the IGF binding activity of IGFBP-3 by proteolytic cleavage causes a dysregulation of the IGF signaling pathways in prostate cancer cells, likely leading to induction of cell growth. These examples illustrate the existence of a balance between autocrine secretion of IGF ligands, IGFBPs, and proteases that are disrupted in carcinogenesis.

Regulation of IGFBP-3 expression

IGFBP-3 expression was increased in PC-3 cells by treatment with TGF-β and retinoic acid, which are known inhibitors of prostate cell proliferation [165]. Androgen was reported to be an important modulator of IGFBP-3 expression as well. In the rat ventral prostate, castration caused increased expression of IGFBP-2, -3, -4, and -5 with maximum levels reached at 72 hours after castration [79]. The 5α-reductase inhibitor finasteride caused a decrease in rat ventral prostate weight. A concomitant increase in IGFBP-3 expression and decrease in IGF-I and IGF-I receptor expression was also observed suggesting that finasteride may suppress prostate growth by inhibition of IGF signaling pathways [80]. In LNCaP prostate cancer cells, the synthetic androgen methyltrienolone (R1881) caused the downregulation of IGFBP-3 mRNA while retinoids (all-*trans*- and 9-*cis*-retinoic acid) caused upregulation. These data suggest that the regulation of IGFBP-3 by nuclear steroid hormone

receptors may be complex and receptor specific [73]. In contrast, dihydrotestosterone and 1,25-dihydroxyvitamin D_3 in combination increased IGFBP-3 production by cultured LNCaP cells [166]. Increased IGFBP-3 was shown to stimulate DNA synthesis and cell proliferation in LNCaP cells. In the CWR22 human prostate cancer xenograft model, IGFBP-3 mRNA did not change significantly after castration or with testosterone treatment and was not increased in the recurrent tumor [77]. Therefore, studies in cell lines, rat ventral prostate and human prostate cancer xenografts gave somewhat disparate results. Nonetheless, the data suggest that IGFBP-3 function is important for prostate cancer growth (in a positive or negative manner).

Clinical significance of IGFBP-3

Circulating IGF-I levels were significantly related to prostate cancer risk while serum IGFBP-3 showed a small inverse association with risk that was stronger in older men [55]. In a second study from Sweden, IGFBP-3 was not associated with prostate cancer risk [56]. Serum IGFBP-3 levels were shown to differ significantly in Caucasian vs. African-American prostate cancer patients with a mean IGFBP-3 plasma level that was 427 ng/ml lower in African-American men [167]. This lowered circulating IGFBP-3 could lead to greater bioavailability of IGF-I, possibly resulting in increased proliferation of prostate cancer cells.

IGFBP-4

Introduction

The gene for IGFBP-4 is located on chromosome 17 [102] and encodes a protein of 24 kDa or 28 kDa when glycosylated [20]. IGFBP-4 has been found in bovine and human fibroblasts [72, 168, 169], endothelial cells [170], spinal cord [171], osteoblasts and bone mesenchyme but not condensing mesenchyme [172-175], muscle [176] and prostate [79, 177, 178] among others. Further, IGFBP-4 has been found in lung cancer [179], neuroblastoma cell lines [127, 140], breast cancer cells [121, 180], osteosarcoma cell lines [175, 181] and prostate [73, 82, 178]. Therefore, IGFBP-4 is expressed in a number of tissues and cell lines of diverse origin having specific patterns of expression throughout development. Of particular interest to prostate cancer is the expression and regulation of IGFBP-4 in bone-derived cell lines and the impact on prostate cell proliferation.

IGFBP-4 Modulation of IGF Action and Expression in Prostate

IGFBP-4 appears to inhibit most of the actions of IGF in vitro [19, 20]. The overexpression of IGFBP-4 in myoblasts prevents IGF-1 mediated myotube differentiation [176]. The removal of IGFBP-4 from the conditioned media of a colon cancer cell line by the addition of antibodies against IGFBP-4 stimulated mitogenesis [182]. Similarly, the addition of exogenous IGFBP-4 directly prevented IGF-1 or -2 binding to the IGF1R in a dose-dependent manner using human osteogenic sarcoma cell lines [183]. Exogenous IGFBP-4 decreased the mitogenic response of vascular smooth muscle cells to IGF-1 [184]. Noll et al. [179] showed that IGF-1 increased growth in non-small cell lung carcinoma cell lines. Furthermore, the IGF effects were directly associated with the degradation of IGFBP-4. Indirect evidence for the inhibitory role of IGFBP-4 comes from studies on the P69 lineage from Plymate et al. [88]. This study found IGFBP-4 expressed in primary cultures of prostate epithelial cells but progressively reduced expression in immortalized and aggressively tumorigenic cell lines derived from the primary cultures. A direct examination of the effects of IGFBP-4 expression in prostate model systems has not been described to date. In summary, every

study to date demonstrates an inhibitory effect of IGFBP-4 on IGF-mediated events.

In prostate cancer cell lines (Table 1) IGFBP-4 protein was found in Du145 [86, 87] and PC-3 [86] conditioned media. In the study by Kimura st al. [86] LNCaP cells had no IGFBP-4 as ascertained by western ligand blot and an abnormally large band in western blot. Goosens et al. [73] showed very weak expression of IGFBP-4 mRNA in LNCaP cells. As discussed above, P69 and the metastatic derivative, M12, continue to express IGFBP-4 in vitro, albeit at lower levels than in primary cultures of prostate epithelial cells [88]. Similarly, Cohen et al. [89] demonstrated the expression of a 24 kDa IGFBP in primary cultures of prostate epithelial cells that is probably IGFBP-4. Primary cultures of prostatic stromal cells also express IGFBP-4 [91]. However, the levels of IGFBP-4 secreted were variable with no detectable trend in expression between normal, BPH and cancerous stromal cells. Overall, these data would indicate a decrease in the expression of IGFBP-4 in accordance with the aggressiveness of the prostatic neoplasm, but a direct inhibitory role has not been demonstrated.

The expression of IGFBP-4 in prostate tissues (Table 2) has not been as extensive as for other IGFBPs. The expression of IGFBP-4 mRNA has been documented in both BPH and prostate cancer [93, 94, 177, 178]. These studies show that IGFBP-4 mRNA is located in both the epithelial and stromal compartments, but the epithelial component expresses much more than the stromal compartment. The expression levels increased slightly from normal and BPH tissue when compared to cancer [94], but this association does not seem to hold with increasing Gleason grade [93]. Therefore, the modest increases in IGFBP-4 protein expression and negative growth effects are probably exceeded by the increases observed for IGF-2, IGFBP-2, and -5 positive growth signals [93-95, 98, 185].

Proteolysis of IGFBP-4

The degradation of IGFBP-4 appears to be a common mechanism to decrease its levels and prevent the inhibitory effects of this binding protein on IGF action. In osteosarcoma cells estradiol increases IGFBP-4 proteolysis [186]. Human fibroblasts, osteosarcoma, and lung cancer all secrete IGFBP-4 proteases in response to IGF [179, 187]. That this activity was EDTA sensitive in lung cancer cell lines indicates the involvement of a matrix metalloproteinase (MMP). Similar indications of MMP involvement in IGFBP-4 degradation were found in pregnancy serum [20, 188-190]. Prostate specific antigen was shown to cleave IGFBP-4 and -5 in seminal plasma [191]. Interestingly, another kallikrein family member related to PSA, gamma-NGF, has demonstrated potent proteolytic activity towards IGFBP-4 [20, 163]. Prostate epithelial cells, but not stromal cells, secrete acid activated proteases [132], probably cathepsins [20, 112], that rapidly degrade IGFBP-4. The degradation of IGFBP-4 decreases the inhibitory action of this IGFBP by liberating bound IGF and preventing further sequestration of IGFs.

Regulation of IGFBP-4 expression

As discussed above, IGFBP-4 inhibits osteosarcoma growth [192]. IGFBP-4 is downregulated by bone morphogenetic protein-7 (BMP-7, OP-1) in osteoblasts and osteosarcoma [193]. BMP-7 and IGF-1 stimulate osteoblasts and osteosarcoma [192, 193] and both are overexpressed in osteosarcoma samples [194]. Furthermore, the mouse prostate expresses BMP-7 in an androgen-dependent manner and LNCaP cells express the receptor for BMP-7 [195, 196]. Given the predilection of prostate cancer to metastasize to the bone and its osteomimetic properties [136], this BMP-7 network would provide an excellent regulatory pathway to suppress IGFBP-4 in prostate cancer cells.

IGF-1 has been shown to decrease IGFBP-4 expression in human fibroblasts [168, 169], colon cancer [197], lung cancer [179], and breast cancer [120]. This is consistent with the increased expression of IGF-2 and negligible change in IGFBP-4 levels in prostate cancer as discussed above. IGFBP-4 expression is associated with cell culture density in vascular smooth muscle cell cultures [184], which may provide the explanation for the decreased proliferative response of these cells to IGF-1 at high density. In contrast to the BMP data, agents that have a differentiative effect in colon cancer, namely estradiol, retinoic acid, and vitamin D, all increase IGFBP-4 levels [197]. This is cell type specific since human osteogenic sarcoma cells, SaOS-2, actually turnover or decrease IGFBP-4 in response to estradiol [186]. Goosens et al. [73] showed that very weak expression of IGFBP-4 mRNA in LNCaP cells was mildly stimulated by androgen. Additional studies on prostate cell lines are not available; however, prostatic involution in rats [79, 177], and humans [82] following androgen ablation results in increased levels of IGFBP-4 mRNA and protein. This would argue that IGFBP-4 is repressed by androgen. Prostate cells have a biphasic growth response to androgens where low and high doses are differentiative, while moderate doses are proliferative [198]. This may explain some of the paradoxical effects of androgens on IGFBP expression.

IGFBP-5

Introduction

The gene for human IGFBP-5 is located on chromosome 2 [102]. IGFBP-5 is a 33-35 kDa protein that migrates as a doublet on sodium dodecyl sulfate polyacrylamide gels, likely due to glycosylation. IGFBP-5 is expressed in a number of tissues and cell lines, including rat osteoblast cells [199], osteosarcoma cells [200], ovarian granulosa cells [201], articular chondrocytes [202], human fibroblasts [203], porcine smooth muscle cells [204], mouse brain [205], rat thyroid [206], and rat mammary gland [207]. IGFBP-5 has also been implicated in prostate cancer progression, but the specific functional role of IGFBP-5 has not been clearly defined. IGFBP-5 is expressed by the rat ventral prostate [79, 81], LNCaP and PC3 cultured cells [112], the CWR22 human prostate cancer xenograft, the Shinogi mouse mammary tumor model [77, 78], as well as benign and malignant human prostate tissue [77, 82, 93, 94](see Tables).

Inhibition of IGF-I actions by IGFBP-5

Proteolysis of IGFBP-5 reduces its affinity for IGF-I, thereby modulating the interaction of the binding protein and the ligand [208]. Early work demonstrated that a serine protease secreted by human fibroblasts effectively cleaved IGFBP-5 [208]. Tindall and colleagues showed that endogenous cathepsin D caused hydrolysis of IGFBP-2, -3, -4, and -5 produced by cultured prostate cancer cells [112]. Thrombin, a serine protease present in extracellular matrix (ECM) of human tissues, degraded only IGFBP-5 [209]. IGFBP-5 binds to the ECM; however, not all of the ECM proteins involved have been identified. Plasminogen activator inhibitor-1 (PAI-1) is a constituent of the ECM, and it was shown to bind IGFBP-5 and partially protect it from proteolysis [210]. Preventing IGFBP-5 binding to the ECM inhibited DNA synthesis in response to IGF-I in smooth muscle cells [211]. An excess of intact IGFBP-5 also inhibited IGF-I action in another study of smooth muscle cells and human osteosarcoma cells [200, 212].

Stimulation of IGF-I actions by IGFBP-5

IGFBP-5 potentiates IGF-I action in smooth muscle cells if proteolytically degraded, although the fragments have low

affinity for IGF-I [203]. A fragment of IGFBP-5 purified from cultured U2 osteosarcoma cells augmented the mitogenic effect of IGF-I on cultured mouse osteoblasts [213]. Gleave and colleagues demonstrated that overexpression of IGFBP-5 in LNCaP cells increased growth rates in the presence and absence of IGF-I [214]. Likewise, treatment of Shinogi mouse mammary tumor cells with IGFBP-5 antisense oligonucleotides inhibited growth of the cells, and this could be reversed by IGF-I treatment [215]. Jones et al. [203] described the IGF potentiating effects of ECM-associated IGFBP-5 in human fibroblasts. These findings suggest that the antiapoptotic and growth-promoting effects of IGF-I could be modulated by IGFBP-5 in prostate cancer progression and that IGF-I activity could be enhanced through upregulation of IGFBP-5, probably via paracrine interactions with adjacent stroma.

Regulation of IGFBP-5 and Expression in Prostate

Previous studies on the regulation of IGFBP-5 have been confined largely to cell lines. Both IGF-I and IGF-II stimulate IGFBP-5 expression [121, 169, 216]. In some cell types, IGF-I increases secretion of IGFBP-5 protein without detectable changes in mRNA [121, 169, 199, 202]. In other cell types [199, 216] both the mRNA levels and secretion of IGFBP-5 are increased. Testosterone was shown to enhance the stimulatory effect of IGF-I on IGFBP-5 mRNA and protein in cultured human genital skin fibroblasts [72]. IGF-I was shown recently to regulate IGFBP-5 gene expression through the phosphatidylinositol 3- kinase, protein kinase B/Akt, p70 S6 kinase signaling pathway in vascular smooth muscle cells [217]. IGFBP-5 expression was induced in cultured rat osteoblasts by 1α, 25-dihydroxyvitamin D3 and parathyroid hormone [218]. IGFBP-5 production was increased fifty-fold in milk after 2 days of rat mammary gland involution induced by removing the suckling pups. This increase was inhibited by 90% if prolactin was administered to the rats. IGFBP-5 synthesis was unaffected by growth hormone, progesterone, or corticosterone in rat mammary glands [207]. Sunic et al. demonstrated that IGF-I and interleukin-1α synergistically increased IGFBP-5 levels in ovine articular chondrocytes, with IGF-I inhibiting proteolysis and interleukin-1α stimulating expression [202].

The regulation of IGFBP-5 expression and function in prostate cancer has been explored using cell lines, xenografts and human tissue. Transforming growth factor β-2 increased IGFBP-5 expression in the PC-3 cell line, whereas retinoic acid treatment resulted in downregulation of IGFBP-5 mRNA [165]. IGFBP-5 transcripts were upregulated rapidly in the rat ventral prostate with peak expression at 72 h after castration [79]. Protein levels for IGFBP-5 in the rat ventral prostate reached maximum expression levels at 9 days after castration, as determined by immunostaining [81]. The association of IGFBP-5 expression with apoptosis caused by androgen withdrawal has been postulated, but a direct functional link has not been described. Increased expression of IGFBP-5 mRNA was associated with apoptotic regression of Shinogi mouse mammary tumors following castration [78]. In contrast, while IGFBP-2 and -4 were increased significantly in involuting prostates of men taking the 5α-reductase inhibitor finasteride, IGFBP-5 protein was not increased and did not co-localize with apoptotic markers [82]. IGFBP-5 mRNA, protein expression and ligand binding activity were decreased following castration in the CWR22 human prostate cancer xenograft model. These were elevated, however, in recurrent CWR22 growing in castrated mice [77], suggesting an association of IGFBP-5 function with progression of prostate cancer. Gleave and colleagues

confirmed these findings in both LNCaP tumors and in the Shinogi model where the overexpression of IGFBP-5 resulted in a faster progression to androgen independence and the growth of tumor cells could be inhibited by treatment with IGFBP-5 antisense oligonucleotides [215]. Therefore, while IGFBP-5 expression appears to be responsive to androgen status in prostate cancer cells and tumors, the direct functional role of IGFBP-5 is not clearly understood.

A possible clue to IGFBP-5 function in benign and malignant human prostate relates directly to the subcellular localization of the mRNA and the protein (Table 2). Stromal-epithelial cell interactions are likely important in normal prostatic development and in the progression to prostate cancer [219]. Plymate and colleagues demonstrated immunostaining for IGFBP-5 protein in epithelium and stroma of benign tissue with increased expression in malignant compared to benign epithelium. The mRNA was localized to the stroma surrounding the acinar glands in benign tissue, prostatic intraepithelial neoplasia, and prostate cancer [94]. Likewise, IGFBP-5 mRNA was localized to the stromal cells of benign prostate and prostate cancer with IGFBP-5 protein expression found exclusively in epithelial cells. Previous studies support these data [91, 220]. There was a 6 to 8-fold increase in protein expression in androgen-dependent and recurrent prostate cancer compared to benign tissue [77]. These findings suggest that IGFBP-5 mRNA is transcribed in the stromal compartment in prostate tissue, but the mRNA is translated into protein that is sequestered by the epithelial cells. Expression of IGFBP-5 by the epithelial component may then enable these cells to bind IGF-I, resulting in mitogenic effects causing cell proliferation. Increased IGFBP-5 protein expression in prostate cancer epithelium would thereby enhance cell proliferation and, in the recurrent state, enable cells to grow in the absence of testicular androgen.

IGFBP-6.

Introduction

The human gene for IGFBP-6 is located on chromosome 12 [102] and encodes a protein of 34 kDa [20, 53]. IGFBP-6 is an O-glycosylated protein which, unlike the IGFBP-1 to -5, binds IGF-2 preferentially [221]. In normal tissue or non-transformed cells, IGFBP-6 has been found in osteoblasts [193, 222], ovarian tissue [19], fibroblasts [223] and primary cultures of prostate epithelial cells [88]. In neoplastic tissues or cell lines IGFBP-6 has been found in neuroblastoma [127, 140], osteosarcoma [37], breast cancer [121], and prostate cancer cell lines and tissues [73, 76, 86-88, 93, 94, 177].

IGFBP-6 Modulation of IGF Action and Expression in Prostate

Due to its extraordinarily high affinity for IGF-2 [221], IGFBP-6 does not affect IGF-1 mediated events. In contrast, excess IGFBP-6 has been shown to decrease IGF-2 mediated DNA synthesis in osteoblasts [221, 223]. IGFBP-6 is induced by IGF-2, which then sequesters IGF-2, thereby dampening the IGF-2 signaling in a negative feedback loop [127, 223]. There is one report of IGFBP-6 stimulating the growth and survival of osteosarcoma cells without binding to the cells or being degraded [37], while similar effects were not observed using normal rat osteoblasts as targets. IGFBP-6 has been found in PC-3, DU145, ALVA-31 and P69 prostate cell lines [76, 86, 88] but not in LNCaP cells [73, 86] (Table 1). Interestingly, as the P69 cell lines become more aggressive they lose expression of IGFBP-6 [88]. Similarly, IGFBP-6 is expressed in PC-3 and DU145 prostate cancer cell lines that have an autocrine proliferation loop involving IGF-2 [86]. Therefore, IGFBP-6 expression may be an attempt by these cells to slow their growth.

In human prostate tissues (Table 2) IGFBP-6 mRNA was found in normal, BPH and prostate cancer [73, 93, 94]. IGFBP-6 has also been found in the rat ventral prostate using cDNA microarrays [177]. Several studies have analyzed the expression of IGFBP-6 in prostate adenocarcinoma versus BPH and PIN [73, 93, 94]. Overall, there was no difference in IGFBP-6 expression among benign and adenocarcinoma tissues, regardless of Gleason grade [93], although both the mRNA and protein were expressed predominantly in the epithelium [94]. It is interesting to speculate that IGFBP-6 levels correspond to the observed levels for IGF-2 expressed in these prostate neoplasms [95, 98]. Additional efforts will also have to determine whether IGFBP-6 has a similar affinity for the large forms of IGF-2 that were shown to be more potent mitogens in cancer [97, 98].

Proteolysis of IGFBP-6

Gamma-NGF, a PSA-related kallikrein, demonstrated potent proteolytic activity towards IGFBP-6 [20, 163]. While, gamma-NGF may synerigize with the IGF system by binding to its own receptor and degrading IGFBP-6 to release IGF-2, it is not expressed in the prostate [15]. Therefore, degradation of IGFBP-6 appears to be a minor mechanism regulating bioavailable IGF-2.

Regulation of IGFBP-6 Expression

IGF-2, as discussed above, upregulates the expression of IGFBP-6 in [127, 223]. The upregulation of IGF-2 appears to be the mechanism behind the upregulation of IGFBP-6 by retinoic acid in neuroblastoma cell lines [127, 140]. While retinoic acid likewise increases IGFBP-6 expression in estrogen receptor negative breast cancer cells, it remains unclear whether this is also mediated by increased IGF-2 expression [121]. Members of the TGF-ß family of proteins downregulate IGFBP-6 expression in osteoblasts [193, 222]. At least in one study this could be directly attributed to transcriptional suppression [222]. However, this same study showed that TGF-ß1 also downregulated IGF-2, which, as discussed, is a potent positive regulator of IGF-2. These effects are consistent with the effects of TGF-β1 to inhibit osteoblast growth and promote differentiation. This seems to be the case for BMP-7, also known as osteogenic protein 1 [193]. BMP-7 also dramatically suppresses IGFBP-6 levels, thereby promoting osteoblast differentiation. The mouse prostate expresses BMP-7 [195] and prostate cell lines express TGF-β receptors [16, 196]. The suppression of IGFBP-6 through this mechanism would allow for prostate cancer cells to respond to the bioavailable IGF-2. In contrast, other differentiation promoting compounds (e.g. Vitamin D) have also been shown to increase IGFBP-6 levels in ALVA prostate cancer cells [76]. This may provide a mechanistic basis for the function of this class of compounds in the prostate. Other steroid hormones may also play a role in the regulation of IGFBP-6 in the prostate. Bruyninx et al. [177] recently showed that IGFBP-6 mRNA is upregulated during castration of the rat ventral prostate. The implication is that androgenic steroids play some role in the suppression of IGFBP-6.

mac25/IGFBP-rP1.

Introduction

The gene for mac25 is located on chromosome 4q12-13 [224] and encodes a protein of 27-31 kDa non-reduced [225] or about 37 kDa when reduced [226]. This protein was cloned by several groups simultaneously and was found to be a unique IGFBP-related protein (IGFBP-rP1) having significant homology to follistatin [224, 227]. Mac25 was cloned from normal lepto-meningial and mammary epithelial cells; its expression was found to be decreased in the corresponding tumor cells [228, 229]. The protein is able to bind IGF with a weak

affinity [230]. During the same period, two other proteins were purified that have been determined to be the same as the protein encoded by mac25. TAF (tumor adhesion factor) was isolated from diploid fibroblasts [231], while PSF (prostacyclin-stimulating factor) was purified from human bladder carcinoma cells [232]. Finally T1A12 was identified by subtractive cDNA cloning using RNAs from a normal breast epithelial cell line Hs578Bst and the breast cancer cell line Hs578T [233]. These were all ultimately found to be the same gene.

Regulation of mac25/IGFBP-rP1 expression

Originally, the expression of mac25 was thought to be ubiquitous due to the presence of mac25 RNA in all tissue samples examined [230]. However, an examination of the protein expression in human tissues undertaken by Degeorges et al. [226] clearly demonstrated that the ubiquitous expression in most tissues was due to strong expression in peripheral nerve cells. In addition, all smooth muscle cells were positive, as were most endothelial cells. Finally, there were specific patterns of immunoreactivity in ciliated structures, kidney tubules, and central nervous system. Immunoreactivity was demonstrated in normal mammary epithelium but not in breast adenocarcinomas, leading to the hypothesis that IGFBP-rP1 might have a tumor suppressor-like function in breast [224]. Chromosomal loss of heterozygosity (LOH) of chromosome 4 in breast cancer accompanies the loss of mac25 expression and lends some support to this theory. In contrast to these reports for breast, studies on mac25 expression in myogenesis show that mac25 levels are highest in proliferating myoblasts and lowest in differentiated myotubules [234]. Indeed, constitutive elevation of mac25 in myoblasts prevents differentiation into myotubules and alters myoblast response to IGF-1 [106]. In support of this, TGF-β, which prevents myoblast differentiation, elevates mac25 levels, while IGF-1, which stimulates myotubule formation, decreases mac25 expression [234]. Finally, the expression of mac25 by breast cancer cell lines is mixed with some aggressive breast cancer cell lines expressing high levels of mac25 mRNA [228, 230]. The normal prostate, in direct contrast to results observed in breast tissue, did not display any positive immunoreactivity in glandular epithelium and only stochastic expression in the basal cell layer. We then stained a cohort of benign prostatic hyperplasia, prostate adenocarcinoma and metastases [235]. Immunoreactivity for IGFBP-rP1 was absent from normal epithelium and BPH epithelium but was increased in prostate carcinoma and metastases. These studies also showed that prostate cancer cell lines that do not express mac25 in vitro have the ability to re-express mac25 when grown in vivo [235]. In contrast, Sprenger et al. [236], in an aggressive, SV40 T-antigen immortalized prostate cell line line, M12, recently demonstrated that overexpression of mac25 decreased cell growth and tumor formation. Therefore, the role of mac25 in differentiation and tumorigenesis will require additional study.

SUMMARY AND PERSPECTIVE

Prostate cancer research is focused on characterizing the mechanism by which prostate cancer develops initially and the process of progression of tumors from the androgen-dependent to the recurrent state. Determining the role of the IGF axis in prostate cancer progression is an important component of this research. This chapter has described the current understanding of the IGF axis, including detailed descriptions of the IGF-1 and IGF-2 ligands, the receptors and the IGF binding proteins. Recent studies describing a strong association of elevated plasma IGF-1 with an increased risk of prostate cancer [55, 56] emphasize the critical role that IGF-1 signaling may have in prostate

cancer growth. Overall, the published data support the idea that IGFs promote growth of prostate cancer cells, while the IGFBPs generally inhibit growth. The roles of cognate receptors and proteases in modulating IGF/IGFBP activity must be taken into consideration as conclusions about the IGF axis are put forth. During the development of prostate cancer the inhibitory IGFBP's (IGFBP-3, -4, and -6) are downregulated at the protein level, while the stimulatory IGFBPs (IGFBP-2 and -5) are upregulated. These changes are accompanied by increases in IGF-2 by both stromal and epithelial components. All these changes effectively increase the growth rate and survival of prostate cancer cells. Several questions can be posed to foster further exploration of the IGF axis in the prostate. Are other components of the IGF axis in the prostate yet to be discovered? Does cross-talk between growth factor signaling pathways occur? Is elevated serum IGF-1 causative for prostate cancer or do prostate tumors produce IGF-1? Is the IGF axis just one of multiple functional components leading to prostate cancer and how do these different pathways interact? Answering these questions will provide important insights into our understanding of prostate cancer development and progression and will encourage the design of new therapeutic strategies to treat this disease.

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2. **1993 NIH/Urology Training Grant Fellowship** (MD Anderson Cancer Center, Houston TX)
3. **1995 American Urologic Association Essay Contest, First Prize** entitled, "Androgen-independent and osseous metastatic progression of human prostate cancer: model development and roles of osteopontin." (submitted by Thalmann, GN et al)
4. **1999 Wendy Will Case Cancer Fund, Inc.**: Research grant to study the immunobiology of PAGE-1 (GAGE-B1) in prostate cancer. (25K for 1 year)
5. **1999 Department of Defense Prostate Cancer Research Program New Investigator Award** DAMD-17-00-1-0049, UG311, An oncofetal marker lost with prostate cancer progression. (75K per year for 3 years support)
6. **2000 SBUR/Merck Young Investigator Award**

7. **2002 NIH/NIDDK** 1R01DK/ES59146-01A1 "Tracking Urogenital Sinus Cell Progeny", (250K/yr for 3 yrs)

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11. Chen M.E., Lin S.-H., Chung, L.W.K. and **Sikes R.A.***, Isolation and characterization of PAGE-1 and GAGE-7: novel genes expressed in the LNCaP prostatic carcinogenesis model that share homology with melanoma associated antigens., J. Biol. Chem. 273 (28): 17618-17625, 1998. ***Corresponding Author**

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13. Theodorescu, D.T., Frierson H.F. and **Sikes R.A.***, Molecular determination of surgical margins following radical prostatectomy., J. Urology 161: 1442-1448, 1999. ***Corresponding Author, Lead Article.**

14. Degeorges A., Wang F., Frierson Jr. H.F., Seth A., Chung LWK , and **Sikes R.A.***, Human prostate cancer expresses the low affinity insulin-like binding protein, IGFBP-rP1., Cancer Res. 59, 2787-2790, 1999. ***Corresponding Author**

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17. Thalmann G.N., **Sikes R.A.**, Wu T.T., Degeorges A, Chang S.-M., Ozen M., Pathak S., and Chung L.W.K., The LNCaP progression model of human prostate cancer: androgen-independence and osseous metastasis, Prostate, 44: 91-103, 2000.

18. Degeorges A., Wang F., Frierson Jr. H.F., Seth A., and **Sikes R.A.***, Distribution of IGFBP-rP1/mac25 in Normal Human Tissues. J . Histochem. Cytochem. 48: 747-754, 2000. ***Corresponding Author**

19. Edlund M., Miyamoto T., **Sikes R.A.**, Ogle R., Laurie G.W., Farach-Carson M.C., Otey C.A., Zhau H.E., and Chung L.W.K., Integrin expression and usage by prostate cancer cell lines on laminin substrata., Cell Growth Differentiation 12 (2): 99-107, 2001. ***Cover**

20. Feldman S.A., **Sikes R.A.**, Eckhoff G.A., Comparison of the Deduced Amino Acid Sequence of Guinea Pig Adenovirus Hexon Protein With That of Other Mastadenoviruses. J. Comparative Med. 51 (2): 120-126, 2001.

B. Articles in Preparation for Submission

1. Fasciana F., van der Made ACJ, **Sikes, RA**, van Weerden WM, Chung LWK, and Trapman J., A urogenital sinus cDNA (ugs148) with a specific expression pattern in embryonic and adult mouse tissues and in human prostate cancer xenografts, (Submitted Cancer Res. 6/00).

2. Kim J.H.,Retief J.D., Fasciana C., Wang F., Chung L., Trapman J., Chung L.W.K., and **Sikes R.A.***, The LNCaP human prostate cancer progression model differentially expresses fetal urogenital sinus-derived sequences (In preparation). ***Corresponding Author**

3. Kao C., **Sikes R.A.**, Zhau, H.E., Li W., Huang K., Chan J., Sim R., Chang S.-M. and Chung L.W.K., p157, An osteosarcoma conditioned media-derived protein that promotes prostate cancer cell growth is human complement Factor H. (In Preparation)

4. Koeneman K.S., Yeung F., Law A., **Sikes R.A.**, Kao C, Nelson J. and Chung L.W.K., Transcriptional down regulation of PSA and enhanced growth of prostate cancer cells by osteoblastic cells., (In Preparation)

5. **Sikes R.A.***, Koeneman K.S., Edlund NM, Bissonette E.A., Nicholson B.E., Bradley MJ, Pienta K.J. amd Chung L.W.K., Cellular interactions in the tropism of prostate cancer to bone., (In Preparation) ***Corresponding Author**

6. Koeneman K.S., **Sikes R.A.**, Yeung F., and Chung L.W.K. Regulation and expression of BSP in human prostate cancer cell lines.

7. Tennant, M, Sprenger C.C., **Sikes RA,** Hwa V., Rosenfeld R.G., and Plymate SR, Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1/mac25) is reduced in human prostate cancer compared to benign prostate epithelium (Submitted "the prostate").

8. Guo N., Ye J.-J., Liang S.-J., Mineo R., Li S.-L, Plymate SR, **Sikes RA**, Fujita-Yamaguchi Y.,, Hammerhead Ribozyme targeted to the human insulkin like growth factor-II: effects of intracellular expression on prostate and breast cancer cell growth. (In preparation, Int. J. Cancer 2001)

C. Published Articles and Book Chapters by Invitation

1. **Sikes R.A.**, TRANSFORMATION STUDIES of a NONTUMORIGENIC RAT PROSTATIC EPITHELIAL CELL LINE with ACTIVATED *MYC* and *NEU* ONCOGENES. Doctoral Dissertation. The University of Texas Graduate School of Biomedical Sciences, Houston, TX. May 1993.

2. He X-Y, **Sikes R.A.**, Thomsen S., Chung L.W.K., and Jacques S.L. Photodynamic therapy-induced programmed cell death in carcinoma cell lines. Proceedings of SPIE, 1993.

3. Zhau H.Y.E., **Sikes R. A.**, Chung L.W.K., The c-*erb*B-2 oncogene in human prostate cancer growth and progression, pp 207-220 in:Protooncogenes and growth factors in steroid hormone -induced growth and differentiation., Ed. Khan, S.A.and Stancel, G.M., CRC press, Inc., Boca Raton, 1994.

4. **Sikes R.A.**, Kao C and Chung L.W.K., Autocrine and Paracrine Mediators for Prostate Growth and Cancer Progression, In: Advances in Urology. Ed. E.J. McGuire, Mosby Year Books, Inc., Chicago, Volume 8, Chapter 2, pp21-60, 1995.

5. Chung L.W.K., Kao C., **Sikes RA**, and Zhau, H.E., Human Prostate Cancer Progression Models and Therapeutic Intervention, Acta Urol. Jap., 43: 815-820, 1997.

6. Gardner T.A., Ko S.-C., Kao C., Shirakawa T., Cheon J., Gotoh A., Wu T.T., **Sikes R.A.**, Zhau H.E., Cui Q., Balian G. and Chung L.W.K., Exploiting stromal-epithelial interaction for model development and new strategies of gene therapy for prostate cancer and osteosarcoma metastases (review), Gene Ther. Mol. Biol. 2: 41-58 , 1998.

7. **Sikes RA**, Molecular epidemiology of cancer: the future is now., Cancer Research Alert 1(2): 13-15, 1999.

8. Cinar B and **Sikes RA,** Control of Steroid Hormone Receptor Action., Cancer Research Alert 1(9): 100-102, 2000.

9. Gregory CW, Degeorges A and **Sikes RA***. The role of the IGF axis in the development and progression of prostate cancer. In Recent Research Developments in Cancer Volume 3, Part II, 3: 437-462, 2001, (Eds) Pandalai.S.G., Mukhtar H and Labrie F, Transworld Research Network, Kerala, India., * **Corresponding Author**

D. Abstracts Accepted for Presentation/Published (*Presented by Dr. Sikes)

1. **Sikes R.A.**, Petrow V., and Chung L.W.K., Effects of antiandrogen, antiestrogen, and/or 5a-reductase inhibitors on mouse prostatic hyperplasia induced by fetal urogenital sinus implant., Organ Systems Program Workshop, Stromal-Epithelial Interactions, April 16-18, Rockville, MD, 1986.

2. **Sikes R. A.**, and Chung, L.W.K., Transformation of rat prostatic epithelial cells with activated *myc* and *neu* oncogenes., American Urologic Association, Inc., Podium Session (AUA) 84th Annual Meeting, Dallas, TX, J. Urol. 141: 218A, 1989.

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4. **Sikes R. A.**, and Chung L.W.K., *Neu* oncogene associated changes in gene expression: correlation with *neu* overexpression and tumorigenicity in rat ventral prostate epithelial cells., Podium Session, Society for Basic Urologic Research, Fall Symposium, Rochester, MN, 1991.

5. Wu H.-C., Hsieh J.-T., Gleave M.E., **Sikes R. A.**, Logothetis C.J., and Chung L.W.K., Combination therapy with pseudomonas exotoxin fusion protein (TP40) and retinoic acid in human transitional cell carcinoma ell lines., American Urologic Association, Inc., (AUA) 87th Annual Meeting, Washington, D. C., J. Urol., 147: 404A, 1992.

6. **Sikes R.A.** and Chung L.W.K., Neu associated changes in gene expression: correlation with neu overexpression and tumorigenicity in rat prostate epithelial cells., 1992 International Symposium on Biology of Prostate Growth, Bethesda, MD, 1992.

7. Zhou J., **Sikes R.A.**, and Zhau H.Y.E., The role of *neu* oncogene in prostatic epithelial carcinogenesis., Society for European Urologic Research, Trento, Italy, 1992.

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9. He X-Y, **Sikes R.A.**, Thomsen S., Chung L.W.K., and Jacques S.L., Photodynamic therapy-induced programmed cell death in carcinoma cell lines. Proceedings of the SPIE, Los Angeles, CA, 1993.

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12. Thalmann G.N., **Sikes R.A.**, Hall M.C., Devoll R.E., Farach-Carson M.C., von Eschenbach A.C., and Chung L.W.K., Implications of bone matrix protein osteopontin (OPN) on prostate cancer progression and osseous metastasis, Society for Basic Urologic Research (SBUR), San Francisco, CA, May 13-14, 1994.

13. **Sikes R.A.**, Kao C. and Chung L.W.K., p157, An osteosarcoma conditioned media-derived protein that promotes prostate cancer cell growth, is human complement factor H., AACR special conference: Basic and Clinical Aspects of Prostate Cancer, Palm Springs, CA Dec. 8-12, 1994.

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15. Thalmann G.N., **R.A. Sikes**, R.E. Devoll, S. Pathak, M.C. Farach-Carson, A.C. von Eschenbach, and L.W.K. Chung, Androgen-independent and osseous metastatic progression of human prostate cancer: Model development and roles of osteopontin., J. Urol. 153 (4): 312A, 1995.

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17. Chen M.E., Shen J.C., Thalmann, G.N., **Sikes R.A.**, von Eschenbach A.C. and Chung, L.W.K., Androgen-independent human prostate cancer progression: the isolation of novel stage-specific sequences using differential mRNA display., American Urological Association (AUA) Annual Meeting, Las Vegas, NV 1995. J. Urol. 153 (4): 267A, 1995.

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21. Thalmann G.N., Troncoso P., Devoll R.E., **Sikes R.A.**, Ball R., Studer U.E., Farach-Carson M.C., and Chung L.W.K., Osteopontin (OPN) in prostate cancer: Biological activity, immunohistochemical and molecular analysis of expression and distribution., Urol. Res., 25(1): 77, O2.3, 1997.

22. Thalmann G.N., **Sikes R.A.**, Chang S.M., Ozen M., Pathak S., and Chung L.W.K., The LNCaP mouse model of human prostate cancer (Pca): androgen-independence and osseous metastasis., Urol. Res. 25(1): 79, O2.14, 1997.

23. Miyamoto T., Wu T.T., Li H., Kao C., **Sikes R.A.**, Zhau H.E., and Chung L.W.K., Prostate growth and PSA expression are affected by stroma-epithelial interaction in collagen gel: stroma cell specificity and the roles of growth fqactors and extracellular matrices. American Urological Association (AUA) Annual Meeting, New Orleans, LA 1997, J. Urol., 157 (Suppl.):

24. Degeorges A., Wang F., Frierson Jr H.F., Seth A., **Sikes R.A.**, Expression of IGFBP-7/mac25 in prostate cancer. Proceedings of the American Association for Cancer Research (AACR) Vol 39, pg 110 # 748 1998 New Orleans, LA.

25. Degeorges A., Wang F., Frierson Jr H.F., Seth A., **Sikes R.A.**, Expression of IGFBP-7/mac25 in prostate cancer. International Symposium on Biology of Prostate Growth, NIH Bethesda MD, 1998

26. **Sikes R.A.**, Kim J.-H., Wang F., Degeorges A., Chung, L.W.K., Fasciana C., Trapman J., Novel fetal urogential sinus gene expression in prostate cancer progression. International Symposium on Biology of Prostate Growth, NIH Bethesda MD, 1998

27. Theodorescu D, Frierson HF, Mills SE, **Sikes RA**, Charlottesville , VA , Molecular determination of surgical margins following radical prostatectomy, American Urological Association (AUA) Annual Meeting, San Diego, CA 1998

28. Koeneman KS ; Yeung F; **Sikes RA;** Chung LWK ; Charlottesville, VA; Nelson JB ; Baltimore, MD Transcriptional down regulation of PSA, and enhanced growth of prostate cancer cells (CaP) by co-culture with osteoblastic cells, American Urological Association (AUA) Annual Meeting, Dallas TX 1999

29. **Sikes RA*** , Koeneman KS. , Bissonnette EA , Pienta KJ and LWK Chung, Cellular Interactions in the Tropism of Prostate Cancer to Bone. Cancer Research at the Millennium. 42nd Annual Clinical Conference, 52nd Annual Symposium on Fundamental Cancer Research, Houston TX Jan 9-12, 2000.

30. Jung CJ, Ou Y-C, Ko SC, **Sikes RA**, Zhau HE, Chung LWK, Differentiation in level of human osteocalcin expression between primary and metastatic prostate tumors. Proceedings of the AACR 41: #258, 91st Annual Meeting of the AACR, SanFrancisco, CA April 1-5, 2000

31. Koeneman KS, **Sikes RA**, Yeung F, Hsieh C-L ,Chung LWK, Sodek J, Adenovirus mediated gene therapy can target metastatic prostate cancer by using the bone sialoprotein (BSP) promoter., American Urological Association (AUA) Annual Meeting, Atlanta GA 2000.

RESEARCH INTERESTS:

1. The IGF axis is comprised of six high affinity binding proteins, IGFBPs 1 to 6, and two high affinity receptors, IGF1R and IGF2R. The IGF1R is a heterodimeric receptor tyrosine kinase (RTK) and has no other known ligands besides IGF-I and IGF-II. The IGF2R has no known signaling function and it binds both IGF-II and mannose-6-phosphate with high affinity. The IGF2R has a reduced affinity for IGF-I, Alterations in the IGF axis, namely changes in expression levels of various IGFBPs and the IGF receptors, have been implicated in the initiation, development and progression of both benign prostatic hyperplasia (BPH) and prostate cancer (PCa). Research in my laboratory is trying to determine the interplay between the IGF axis and the androgen receptor with particular emphasis on changes that occur during progression of PCa to an androgen independent phenotype. In addition to testing the signaling via the IGF1R, I am testing the responsiveness of the various IGFBPs to androgen in a progression model of human prostate cancer. The aim of this research is to elucidate the mechanism(s) of prostate cell survival and growth during progression to androgen independence. Identification of the components of this system should allow for the development of therapeutic compounds directed at specific molecules involved in the process. Current approaches include the use of retrovirally delivered ER-localized scFv to the IGF1R, ribozymes, and the use of microarray screening to identify candidate genes in the prostatome regulated by both IGFI and androgen. Additional studies are characterizing the role of the IGFBP's in prostate development.

2. Analysis of novel gene expression during prostate gland development and the application of these findings to BPH and malignancy (PCa) or **Onco-fetal translational genomics**. We have cloned the expressed RNAs of mouse urogenital sinus (E15.5-16.5) into bacterial libraries. This project involves: 1) The expansion and direct sequencing of these expressed sequence tags (ESTs) and comparison to international sequence databases to ascertain the uniqueness of each sequence. To date the project has yielded about 52% unique sequences from the 787 ESTs sequenced to date as compared to the GenBank database using FASTA analysis. 2) The unique clones will be narrowed using biochemical and sequence localization techniques to ascertain those with relevance to prostate biology, i.e. prostate development or disease progression. To this end a matrix array of the clones was hybridized to both LNCaP, androgen sensitive PCa, or C4-2, androgen independent PCa, radiolabeled single-stranded cDNAs. This approach has yielded 31 fetal sequences whose expression level changes from LNCaP to C4-2 indicating a potential association with PCa progression. 3) I am now in the process of cloning the full-length open reading frames for some of the ESTs with the largest changes in expression level.

   The examination of the UGS library will be expanded to include an examination of the directed expression of UGS RNAs in a lobe and temporal specific manner. The goal is to determine which UGS genes represent a core of genes required for the development and maintenance of the different lobes of the prostate. This is primarily a bioinformatics project The data will then be examined for their applicability as markers for human prostate tissue. Some of these may have utility in prostate cancer diagnosis, prognosis and/or therapeutic application.

3. Stromal-epithelial interaction: emphasis towards PCa metastasis to bone and the unique behavior/biochemistry of bone-prostate interaction. PCa has the proclivity to metastasize to the bone. About 80% of metastatic prostate cancer will involve the bone. We are currently evaluating this interaction by : 1) developing new techniques to study prostate cancer: bone interaction using the androgen dependent (LNCaP) or androgen independent, LNCaP-derived (C4-2) cell lines. Using these new models we are able to screen new compounds for their efficacy in androgen-dependent and -independent prostate cancer. 2) discovering novel gene sequences associated with prostate cancer progression. By ascertaining the pattern and cell specificity of these genes' expression we hope to gain insight into their function and role in prostate cancer progression and metastasis. Growth, differentiation and signal transduction will be the focus of study for these genes

4. Therapeutic Drug Discovery. Advanced prostate cancer has few effective therapeutic options. Therefore, the need exists to continue the development and testing of novel therapeutic compounds. In collaboration with Dr. Milton Brown, a synthetic and medicinal chemist in the Department of Chemistry at the University of Virginia, my lab has initiated the testing of novel compounds to inhibit prostate cancer growth. Currently testing includes the use of novel sodium channel blockers, na'aladase inhibitors and growth factor inhibitors.

PERSONAL REFERENCES:

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PATENTS (Pending & Filed Provisional)

1. Utilization of osteocalcin promoter to deliver therapeutic genes to osteosarcoma, glioma, melanoma, lung and prostate cancers and their osseous metastases. Chung LWK, Kao C, Ko S-C, Cheon J, and **Sikes RA** (US Patent # 5,772,993).

2. In vivo suppression of osteosarcoma pulmonary metastasis with intravenous osteocalcin promoter-based toxic gene therapy. Chung LWK, Kao C, **Sikes RA,** Ko S-C, Cheon J, and Sung BK, (US Patent # 6,159,467).

3. Isolation and use of fetal urogenital sinus expressed sequences. **Sikes RA**, Kim JH, Fasciana C, Trapman J, and Chung LWK (Provisional #60/085,383 5/14/98, Patent Conversion initiated 6/00, Abandoned 11/01).

4. Detection of residual cancer during radical prostatectomy. Theodorescu D and **Sikes RA** (Provisional #60/095,484 8/13/98)-Declined and dormant.

5. PAGE-1, a prostate cancer associated gene with immunotherapeutic potential. **Sikes RA**, Chen ME, Lin S-H, and Chung LWK, (Provisional Submitted)-Declined by MDACC and UVHSC, recently initiated independently by a Pharmaceutical Company

ADMINISTRATIVE RESPONSIBILITIES

I. Teaching

A. Research Oriented

1. Participation or Direction in the training of Clinical Research Fellows (1987-present):

a. Louis Pisters, M.D. (AFUD scholar) 1991-1993
Current Position: Asst. Professor of Urology, MD Anderson Cancer Center, Houston TX

b. George N. Thalmann, M.D. 1993-1995
Current position: Assoc. Professor of Urology, University of Bern, Bern, Switzerland
c. Michael E. Chen, M.D. (AFUD scholar) 1993-1995
Current Position: Asst Professor of Urology, MD Anderson Cancer Center, Houston TX
d. Tony Wu, M.D. 1995-1997
Current Position: Asst. Professor of Urology, Veterans General Hospital-Kaohsiung,Taiwan
e. Mitchell H. Sokoloff, M.D. (AFUD scholar) 1997-1999
Current Position: Asst. Professor of Urology, University of Chicago, Chicago IL
f. Kenneth K. Koeneman, M.D. (AFUD scholar) 1998-2000
Current Position: Asst. Professor of Urology, U.T. Southwestern, Dallas TX
g. Brian E. Nicholson, M.D. 2000-current (Urology Training Grant)
Current Position: Research Instructor, Dept. of Urology, University of Virginia, Charlottesville VA

2. Urology Resident Research Training:

Jin-Hee Kim, M.D. 1997-1998
Current Position: Urology Resident UCLA Medical School, Los Angeles, CA

3. Post-doctoral Fellows:

a. Armelle Degeorges, Ph. D. 1996-1998
Current Position: Research Scientist at Neurotech S. A., a Genopole Industries, Paris, France
b. Fuan Wang, M.D., Ph.D. 1997-1999
Current Position: Senior Associate Research Scientist , University of Western Ontario, London, Ontario, Canada
c. Chaeyong Jung, DVM, PhD 1998-2000
Current Position: Research Assistant Professor, Indiana University and Purdue University, Department of Urology, Indianapolis, IN

4. Independent Study Undergraduates : UVA Biology 495-496. Independent Study Mentor

a. Nila Rafiq, UVa 3rd year, Biology Major
Term: September 1996-May 1997
b. Thomas-Pierre Phillippe, UVa 3-4th year, French Major
Term: January 1997-December 1997
c. Dannon Smith, UVa 4th year, Biology Major
Term: Sept. 1999-May 2000
d. Todd Phillip Hanson, UVa 4th year, Biology Major
Term : Sept. 2001-May 2002 (joint w/ Dr. Brown)

B. Invited Lectureships and Courses

1. Interim Lecturer (Anatomy and Physiology), Houston Community College, Houston, Texas 1994.
2. Group presentation on, "Prostate cancer progression associated genes." UVa Mini-Medical School 1996.
3. University of Virginia Matrix Group, "Role of Osteopontin in Prostate Cancer Progression." April 1997.
4. IBC's International Symposium on Prostate Cancer: Advances in Diagnostics and Therapeutic Development. Amelia Island Plantation, FL "Isolation and Characterization of Novel Genes Associated with Prostate Cancer Progression" November 1998 and session chair.
5. Centre Nationale de la Recherche Scientifique, Paris ,France, December 10, 1999. "Regulation of IGF Binding Protein Expression in the LNCaP Model of Human Prostate Cancer progression"
6. Department of Urology, University of Bern, Bern, Switzerland, December 13-14, 1999.
   a. "Regulation of IGF Binding Protein Expression in the LNCaP Model of Human Prostate Cancer progression"
   b. "Expression Pattern of IGFBP-rP1 in Normal Human Tissues and Prostate Cancer"
7. Feist-Weiler Cancer Center, LSU Medical Center, Shreveport LA, March 5, 2001. "The IGF axis in prostate cancer progression."
8. Department of Biological Sciences, University of Delaware, Newark DE, May 4, 2001. "The IGF axis in prostate cancer progression."

9. Karmanos Cancer Center and Department of Urology, Wayne State University, Detroit MI, May 9, 2001. "The IGF axis in prostate cancer progression."
10. Man to Man of the Valley, Prostate Cancer Support Group. May 17, 2001, Augusta Medical Center, Fishersville, VA. " The role of insulin-like growth factors in the development and progression of prostate cancer."
11. Department of Biological Sciences, University of Delaware, Newark DE, September 24, 2001. "Extracellular Matrix Interaction in Prostate Cancer Progression."
12. INSMED, Inc., Richmond , VA April 19, 2002 "Ion Channel Therapeutics." Joint presentation with Dr. Milton L. Brown.

C. Mentoring

1. Graduate students & committees

   a. Paul Deeble, B.S., Graduate Student in Microbiology, Served on Thesis Committee since May 2001.
   Advisor: Sarah J. Parsons, Ph.D.
   Defense -March 28, 2002

   b. Genevieve Höll, B.S., Graduate Student in Dept. of Chemistry, Assist Dr. Brown in project design and direct supervision of Ms. Höll.
   Advisor: Milton L. Brown, M.D., Ph.D.

2. Minority Mentoring Program-Office of African American Affairs at UVA. A general undergraduate mentoring program where faculty are meant to serve the students as a confidant and general advisor.

   a. Valerie Carmen Dominique.
   Spring Semester 2001
   Fall Semester 2001 (She took study abroad option this semester. Contact by e-mail only)

II. Peer-Review Process

A. Manuscript reviews (Ad hoc basis).

1. Biology of Reproduction
2. Cancer Research
3. Developmental Dynamics
4. Endocrinology
5. J. Investigative Urology
6. Journal of Biological Chemistry
7. Molecular Carcinogenesis
8. The Prostate
9. Urology
10. British Journal of Cancer

B. Non-NIH Grants Reviewed:

1. Department of Veterans Affairs Extramural Review Program _____(3)
2. MD Anderson Extramural Review of Institutional Grants _____(1)
3. University Grants Committee-Hong Kong _____(3)

C. Study Sections (NIH and DOD)

1. Ad Hoc Member - Program Project Site Visit: PO1 CA84203-01; Hasan, T- PI Boston, MA June 2-4, 1999
2. Ad Hoc Member - NCI Scientific Review Group, Subcommittee C, Program Project Parent Committee, Washington DC, August 10-12, 1999.
3. Full Panel Member- Department of Defense, USAMRMC Prostate Cancer Research Program FY2000, Washington DC, July 6-8, 2000.

4. Full Panel Member- Department of Defense, USAMRMC Prostate Cancer Research Program FY2001, Washington DC, May 20-22, 2001.

III. Editorial Board(s)

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